



UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

GABRIELA NOVO BORGHI

**SOBREPESO E OBESIDADE, COMPORTAMENTO ALIMENTAR
E BIOMARCADORES SALIVARES DO APETITE NA PRIMEIRA
INFÂNCIA: UM ESTUDO LONGITUDINAL**

**OVERWEIGHT AND OBESITY, FOOD BEHAVIOR AND
SALIVARY APPETITE BIOMARKERS IN EARLY CHILDHOOD:
A LONGITUDINAL STUDY**

Piracicaba
2022

GABRIELA NOVO BORGHI

**SOBREPESO E OBESIDADE, COMPORTAMENTO ALIMENTAR
E BIOMARCADORES SALIVARES DO APETITE NA PRIMEIRA
INFÂNCIA: UM ESTUDO LONGITUDINAL**

**OVERWEIGHT AND OBESITY, FOOD BEHAVIOR AND
SALIVARY APPETITE BIOMARKERS IN EARLY CHILDHOOD:
A LONGITUDINAL STUDY**

Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Doutora em Odontologia, área de Odontopediatria

Thesis presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Dentistry, in Pediatric Dentistry area.

Orientador: Profa. Dra. Maria Beatriz Duarte Gavião

Esse trabalho corresponde a versão final da tese defendida pela aluna Gabriela Novo Borghi, orientada pela professora Maria Beatriz Duarte Gavião.

Piracicaba
2022

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Odontologia de Piracicaba
Marilene Girello - CRB 8/6159

B644s Borghi, Gabriela Novo, 1989-
Sobrepeso e obesidade, comportamento alimentar e biomarcadores salivares do apetite na primeira infância : um estudo longitudinal / Gabriela Novo Borghi. – Piracicaba, SP : [s.n.], 2022.

Orientador: Maria Beatriz Duarte Gavião.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Aleitamento materno. 2. Comportamento alimentar. 3. Obesidade pediátrica. 4. Grelina. 5. Leptina. I. Gavião, Maria Beatriz Duarte, 1955-. II. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Overweight and obesity, food behavior and salivary appetite biomarkers in early childhood : a longitudinal study

Palavras-chave em inglês:

Breast feeding

Feeding behavior

Pediatric obesity

Ghrelin

Leptin

Área de concentração: Odontopediatria

Titulação: Doutora em Odontologia

Banca examinadora:

Maria Beatriz Duarte Gavião [Orientador]

Taís de Souza Barbosa

Lenita Marangoni Lopes

Darile Santos Araujo

Lívia Pagotto Rodrigues

Data de defesa: 29-07-2022

Programa de Pós-Graduação: Odontologia

Identificação e informações acadêmicas do(a) aluno(a)

- ORCID do autor: <https://orcid.org/0000-0001-9067-0857>

- Currículo Lattes do autor: <http://lattes.cnpq.br/9074362921015012>



UNIVERSIDADE ESTADUAL DE CAMPINAS

A Comissão Julgadora dos trabalhos de Defesa de Tese de Doutorado, em sessão pública realizada em 29 de julho de 2022, considerou a candidata GABRIELA NOVO BORGHI aprovada.

PROF^a. DR^a. MARIA BEATRIZ DUARTE GAVIÃO

PROF^a. DR^a. TAÍS DE SOUZA BARBOSA

PROF^a. DR^a. DARLLE SANTOS ARAUJO

PROF^a. DR^a. LÍVIA PAGOTTO RODRIGUES

PROF^a. DR^a. LENITA MARANGONI LOPES

A Ata da defesa, assinada pelos membros da Comissão Examinadora, consta no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa da Unidade.

DEDICATÓRIA

Agradeço primeiramente a Deus, por estar sempre me guiando e me dando suporte durante toda minha caminhada.

Dedico essa conquista:

Ao meu marido, Vinícius, por estar ao meu lado em todos os momentos, por sempre se mostrar presente e participativo, além de todo amor e dedicação que tem por mim, tornando a jornada mais leve e tranquila. Assim como toda sua família.

Aos meus pais, Fabiana e Martinho e minha irmã, Rafaela, por todo apoio e incentivo em todos os

momentos da minha vida, por me ajudarem a enfrentar as dificuldades e não me deixarem desistir, por serem minha base, meu alicerce, por todo amor, carinho e compreensão que depositam sobre mim.

A toda minha família por estar sempre torcendo por mim.

Especialmente a minha avó paterna, Maria Lúcia, que faz parte, que vibra e que me apoia em todas as conquistas da minha vida.

Aos meus amigos e amigas que sempre estiveram presentes e que fazem parte de todas as minhas conquistas, mesmo alguns estando longe, por nossa amizade de tantos anos que nos conforta, ampara e nos ajuda a enfrentar todos os desafios.

AGRADECIMENTOS

À Universidade Estadual de Campinas, na pessoa do seu Magnífico Reitor Prof. Dr. Antônio José de Almeida Meirelles. À Faculdade de Odontologia de Piracicaba, na pessoa do seu Diretor, Prof. Dr. Francisco Haiter Neto. Ao Prof. Dr. Valentim Adelino Ricardo Barão Coordenador do Programa de Pós-Graduação da FOP/UNICAMP. A Profa. Dra. Michelle Franz Montan Braga Leite, Coordenadora do Programa de Pós-Graduação em Odontologia da FOP/UNICAMP.

Às professoras do Departamento de Odontologia Infantil, área de

Odontopediatria, Profa. Dra Fernanda Miori Pascon, Profa. Dra. Regina Maria

Puppini Rontani, Profa. Dra. Maria Beatriz Duarte Gavião e Prof. Dra. Caroline Steiner Alarcon, que contribuíram para meu aprimoramento profissional.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - código de financiamento 001, pelo apoio através da concessão da bolsa de doutorado.

Ao técnico do laboratório de Odontopediatria Marcelo atenção e prontidão em me auxiliar nas análises e em todos os procedimentos realizados no laboratório.

Aos queridos amigos do mestrado e doutorado a amizade de vocês foi fundamental durante esses anos de pós-graduação, vou levar comigo para sempre todos os momentos vividos. Quero agradecer de coração pelo apoio de vocês.

À todos os voluntários e suas respectivas famílias que contribuíram participando da pesquisa, a todas as escolas e creches do município de Piracicaba- SP, e a todas as diretoras, coordenadoras e professoras das escolas, graças a vocês foi possível concretizar esse trabalho.

AGRADECIMENTOS ESPECIAIS

À Prof. Dra. Maria Beatriz Duarte Gavião, minha orientadora, agradeço pela orientação, respeito, dedicação e por todos os ensinamentos durante os anos do Doutorado.

RESUMO

A prevalência da obesidade infantil no Brasil vem crescendo acentuadamente. O aleitamento materno pode ter efeito protetor contra a obesidade em geral e o comportamento alimentar pode ser um fator influenciador. Biomarcadores salivares como grelina e leptina têm sido utilizados como método diagnóstico para sobrepeso e obesidade. Portanto, este estudo observacional longitudinal teve como objetivo avaliar o comportamento alimentar através dos questionários Baby Eating Behavior (BEBQ) e Children Eating Behavior (CEBQ), a duração do aleitamento materno exclusivo e quantificar os biomarcadores salivares grelina e leptina durante os primeiros 3 anos de vida e possíveis influência no estado nutricional. Um total de 122 crianças de 5 meses a 17 meses foram inicialmente selecionadas e avaliadas por três anos consecutivos. A amostra foi selecionada dentro das creches municipais de Piracicaba - SP. Na primeira fase do estudo, os dados foram obtidos por meio de anamnese. As medidas antropométricas foram avaliadas e os pais/responsáveis foram solicitados a preencher o BEBQ. Após um ano, na segunda fase do estudo, os dados antropométricos foram reavaliados. Na terceira fase, dois anos depois, os dados antropométricos foram novamente verificados e os pais/responsáveis foram solicitados a preencher o CEBQ. Além disso, amostras de saliva foram coletadas para avaliação dos biomarcadores salivares de apetite, grelina e leptina. Os resultados mostraram que a idade média da amostra na primeira fase foi de $12,60 \pm 2,59$ meses. Na primeira, segunda e terceira fases, 39,4%, 41% e 30% das crianças, respectivamente, apresentaram sobrepeso/obesidade. As crianças que foram amamentadas ≥ 6 meses permaneceram eutróficas ao longo do tempo. Em relação ao BEBQ, as crianças eutróficas apresentaram valores significativamente menores para a subescala lentidão na alimentação do que as crianças com sobrepeso/obesidade. O aleitamento materno correlacionou-se positivamente com a responsividade alimentar e ingestão lenta nas subescalas comer, enquanto o percentil e o estado nutricional correlacionaram-se negativamente com a lentidão na alimentação. As subescalas do CEBQ foram semelhantes entre os estados nutricionais. Correlações significativas ocorreram entre as subescalas BEBQ e CEBQ, mostrando que o apetite durante o período de aleitamento tende a se assemelhar às características do comportamento alimentar após dois anos. As crianças com sobrepeso/obesidade apresentaram valores de grelina significativamente mais baixos do que as crianças eutróficas. Para a leptina, não ocorreu diferença significativa entre os grupos. Os valores de grelina foram negativamente correlacionados com percentil e estado nutricional e os valores de leptina foram positivamente correlacionados com a duração do aleitamento materno. Percentil e estado nutricional na

terceira fase e valores de grelina correlacionaram-se negativamente com a responsividade à saciedade (CEBQ), conforme esperado. O peso ao nascer e a leptina correlacionaram-se negativamente com a ingestão lenta (CEBQ). Conclui-se que o estado nutricional mais frequente nas três fases foi eutrófico. As crianças que foram amamentadas ≥ 6 meses permaneceram eutróficas ao longo do tempo, sugerindo um efeito protetor do aleitamento materno. As subescalas dos questionários sobre comportamento alimentar não mostraram diferenças entre os grupos eutrófico e sobrepeso/obeso. Crianças com sobrepeso/obesidade apresentaram valores de grelina salivar significativamente menores do que crianças com peso normal, mostrando que quanto menores os valores de grelina, maior o percentil ou estado nutricional. Para a leptina, não houve diferença significativa entre os grupos. A frequência encontrada de sobrepeso e obesidade no âmbito geral do estudo sugere alimentação não saudável desde os primeiros anos de vida.

Palavras chave: Aleitamento materno. Comportamento alimentar. Obesidade infantil. Grelina. Leptina.

ABSTRACT

The prevalence of childhood obesity in Brazil has been growing sharply. Breastfeeding may have a protective effect against obesity in general and eating behavior may be an influencing factor. Salivary biomarkers such as ghrelin and leptin have been used as a diagnostic method for overweight and obesity. Therefore, this longitudinal observational study aimed to assess eating behavior through the Baby Eating Behavior (BEBQ) and Children Eating Behavior (CEBQ) questionnaires, the duration of exclusive breastfeeding and quantify the salivary biomarkers ghrelin and leptin during the first 3 years of life and possible influence on nutritional status. A total of 122 children aged 5 months to 17 months were initially selected and evaluated for two consecutive years. The sample was selected within the municipal day care centers in Piracicaba - SP. In the first phase of the study, data were obtained through an anamnesis. Anthropometric measurements were evaluated and parents/guardians were asked to complete the BEBQ. After one year, in the second phase of the study, the anthropometric data were reassessed. In the third phase, two years later, the anthropometric data were verified again and the parents/guardians were asked to complete the CEBQ. In addition, saliva samples were collected to evaluate the salivary biomarkers of appetite, ghrelin and leptin. The results showed that the mean age of the sample in the first phase was 12.60 ± 2.59 months. Sixty percent of the parents/guardians had good educational levels. In the first, second, and third phases, 39.4%, 41%, and 30% of the children, respectively, presented overweight/obesity. Children who were breastfed ≥ 6 months remained eutrophic over time. Regarding the BEBQ, the eutrophic children presented significant lower values for slowness in eating subscale than children with overweight/obesity. Negative correlations between BEBQ subscales showed that the increase in food approach traits decreased avoidance traits. Breastfeeding was positively correlated with food responsiveness and slowness in eating subscales, whereas percentile and nutritional status correlated negatively with slowness in eating subscale. CEBQ subscales were similar between nutritional status. Significant correlations occurred between BEBQ and CEBQ subscales, showing that appetite during milk feeding period tended to be like eating behavior traits after two years. Overweight/obese children had significantly lower ghrelin values than eutrophic children. For leptin, no significant difference between groups occurred. Ghrelin values were negatively correlated with percentile and nutritional status and leptin values were positively correlated with the breastfeeding duration. Percentile and nutritional status in the third phase and ghrelin values were negatively correlated with Satiety Responsiveness (CEBQ), as expected. Birth weight and leptin were negatively correlated with slowing in eating (CEBQ). It

is concluded that the most frequent nutritional status in the three phases was eutrophic. Children who were breastfed ≥ 6 months remained eutrophic over time, suggesting a protective effect of breastfeeding. The subscales of the questionnaires on eating behavior did not show differences between the eutrophic and overweight/obese groups. Overweight/obese children had significantly lower salivary ghrelin values than normal weight children, showing that the lower the ghrelin values, the higher the percentile or nutritional status. For leptin, there was no significant difference between groups. The frequency found of overweight and obesity in the general scope of the study suggests unhealthy eating from the first years of life.

Keywords – Breast feeding. Feeding behavior. Pediatric obesity. Ghrelin. Leptin

SUMÁRIO

1 INTRODUÇÃO	13
2 ARTIGO: Overweight and obesity, food behavior and salivary appetite biomarkers in early childhood. A longitudinal study	19
3 CONCLUSÃO	51
REFERENCIAS	52
ANEXOS	57
Anexo 1 – Dados demográficos e história social, anamnese e exame clínico	57
Anexo 2 – Saúde bucal	60
Anexo 3 – Avaliação antropométrica	61
Anexo 4 – Questionário BEBQ	62
Anexo 5 - Questionário CEBQ	64
Anexo 6 – Relatório de verificação de originalidade e prevenção de Plágio	66
Anexo 7 – Comitê de Ética	67
Anexo 8 – Comprovante de submissão do artigo	68

1 INTRODUÇÃO

A obesidade é definida como uma patologia crônica degenerativa, decorrente do acúmulo de tecido adiposo de maneira acentuada, considerada uma doença multifatorial, ou seja, acontece através de uma complexa interação entre fatores como os comportamentais, genéticos, fisiológicos e psicológicos. Dentre esses fatores, destacam-se os fatores ambientais e fatores genéticos (Nascimento et al., 2016). Acredita-se que os fatores ambientais seriam mais relevantes na incidência da obesidade do que propriamente os fatores genéticos.

A obesidade infantil é um problema de saúde pública mundial e segundo a Organização Mundial de Saúde - OMS, os dados mais recentes de 2020, mais de 38,9 milhões de crianças, menores de 5 anos de idade, estão acima do peso. Nos últimos anos foi possível observar um aumento na prevalência da obesidade de uma forma geral, em todas as faixas etárias. O aumento da obesidade infantil no Brasil é preocupante, e a estimativa é que 6,4 milhões de crianças tenham excesso de peso no Brasil e 3,1 milhões já evoluíram para obesidade. (OMS, 2020)

A doença afeta 13,2% das crianças entre 5 e 9 anos acompanhadas no Sistema Único de Saúde (SUS), do Ministério da Saúde, e pode trazer consequências preocupantes ao longo da vida. Nessa faixa-etária, 28% das crianças apresentam excesso de peso, um sinal de alerta para o risco de obesidade ainda na infância ou no futuro. Entre os menores de 5 anos, o índice de sobrepeso é de 14,8%, sendo 7% já apresentam obesidade. Os dados são de 2019, baseados no Índice de Massa Corporal (IMC) de crianças que são atendidas na Atenção Primária à Saúde (SAPS). Segundo o Atlas Mundial da Obesidade e a Organização Mundial da Saúde (OMS), o Brasil estará na 5ª posição no ranking de países com o maior número de crianças e adolescentes com obesidade em 2030, com apenas 2% de chance de reverter essa situação se nada for feito. Segundo o Ministério da Saúde, em 2019, no Brasil, 18,9% das crianças menores de 2 anos tem excesso de peso, 120 mil (7,9%) estão com obesidade e 168 mil (11%) com sobrepeso. Além disso, nos menores de 6 meses avaliados apenas 54% estavam em aleitamento materno exclusivo.

O Ministério da Saúde mostra que o problema de obesidade já afeta 1/5 da população infantil e pode resultar em uma futura geração de hipertensos, diabéticos, problemas renais, cardiovasculares e cerebrais. Segundo a ABESO – Associação Brasileira para o estudo da obesidade e síndrome metabólica, na região sudeste, 38,8% das crianças entre 5 e 9 anos está acima do peso.

Diante disso, muitas pesquisas têm sido desenvolvidas a fim de encontrar quais possíveis mecanismos estão associados com o desenvolvimento da obesidade. Segundo Francis (2015), a partir de estudos sobre epigenética, descobriu-se que existe uma predisposição genética que pode ser influenciada pelos fatores ambientais, que atuam em um período pré-determinado do desenvolvimento infantil, o que poderia causar alterações em determinadas expressões de alguns genes associados a obesidade.

Atualmente, as pesquisas mais recentes, revelam que as primeiras experiências nutritivas podem determinar futuramente a suscetibilidade a doenças crônicas, assim como a obesidade. (Bellodi, 2018). No mesmo sentido, Cunha et al. (2021) destacam que o aleitamento materno está incluso em um período de muita importância no desenvolvimento metabólico infantil, denominado de “primeiros mil dias”. Evidências sugerem que os “primeiros mil dias” poderiam afetar o desenvolvimento do sobrepeso e da obesidade (Pietrobelli et al., 2017) e que o aleitamento materno está inversamente associado ao sobrepeso e obesidade infantil, sugerindo um possível efeito protetor (Qiao et al., 2020).

Nos primeiros anos de vida, o leite materno é o alimento considerado mais adequado ao bebê, representa a experiência nutricional mais precoce do recém-nascido. A Organização Mundial da Saúde (OMS) e o Ministério da Saúde (MS) recomendam aleitamento materno exclusivo (AME) por seis meses e complementado até os dois anos ou mais (BRASIL, 2009). Os benefícios da ingestão de leite materno podem ser observados em longo prazo, dentre os quais se investiga atualmente a sua influência na redução de riscos de doenças crônicas, como obesidade, diabetes e hipertensão arterial. Entre os benefícios da amamentação, Perrine et al. (2014) verificaram que esta associou-se com comportamento alimentar saudável aos 6 anos de idade, consistindo fator relevante na avaliação da obesidade e das doenças crônicas na infância.

Jensen et al. (2015) verificaram que a amamentação exclusiva é um fator controlador importante, mas outros fatores, como ganho de peso da mãe no período gestacional e a introdução e composição dos alimentos, podem desempenhar papel relevante no que diz respeito aos padrões alimentares e consequentemente no desenvolvimento do sobrepeso e obesidade. No Brasil foi observado que o consumo de alimentos não saudáveis por crianças abaixo de um ano de idade foi alto, indicando a necessidade do desenvolvimento de estratégias efetivas para implementação da alimentação adequada precocemente, para prevenção da obesidade e outras doenças (Saldiva et al., 2014).

Além disso, foi também observado por Syrad et al. (2015) que fórmulas infantis têm sido utilizadas como substituto de alimentos sólidos e não como complemento alimentar; os autores sugeriram que são necessários estudos para determinar o quanto as fórmulas infantis influenciam o desenvolvimento corporal, o comportamento alimentar e, conseqüentemente, a saúde. Diversas hipóteses vêm sendo levantadas para explicar o motivo pelo qual o aleitamento materno protege a criança contra a obesidade, algumas se referem ao efeito protetor que estaria envolvido na composição específica e única do leite humano e também a fatores ambientais e comportamentais (Pauli, 2013). Diferente do que acontece quando o bebê utiliza fórmulas infantis, quando eles fazem aleitamento materno, eles estipulam a quantidade de leite que desejam consumir, ou seja, se alimentam até sentir-se saciados. Acredita-se que devido a essa característica, o aleitamento materno exclusivo demonstre um possível efeito protetor sobre a obesidade. (Ardic et al., 2019). Sabe-se que o leite materno possui em sua composição substâncias bioativas, como por exemplo a grelina e leptina, que podem ter um efeito protetor contra a obesidade futura, devido a sua ação reguladora do apetite e saciedade (SBP, 2009; Qiao et al., 2020).

Os biomarcadores, leptina e grelina, têm despertado interesse como eventual ligação entre apetite-saciedade e o peso corporal, são sintetizados em diversos tecidos, incluindo as glândulas salivares (Aydin et al., 2005). Segundo Rodrigues e colaboradores em 2017 os estímulos sensoriais produzidos pelo gosto, cheiro, textura e aparência dos alimentos interagem com os sinais de saciedade produzidos pela dilatação do estômago e pela liberação de hormônios da saciedade determinando o prazer e o sabor do alimento, influenciando na escolha do tipo e da quantidade do alimento a ser ingerido. Neste sentido, Sudi et al. (2000) observaram que o IMC é o principal determinante para a variação dos níveis de leptina em crianças e adolescentes obesos. Segundo Arslan et al. (2010), o aumento nos níveis de grelina e a diminuição nos níveis de leptina alteram o padrão de ingestão alimentar, aumentando a sensação de fome e diminuindo a saciedade, respectivamente, resultando em desajustes nutricionais em crianças.

A leptina é o hormônio associado com a regulação da saciedade, da taxa metabólica basal, massa corporal e gasto energético (Guzel et al., 2017), sintetizada principalmente no tecido adiposo. Dessa maneira, pode-se observar que os níveis aumentam de forma proporcional ao aumento de massa gorda; sendo assim, em pessoas obesas, podemos observar um nível elevado de leptina (Mehrdad et al., 2020). Ela atua na redução do apetite e conseqüentemente no controle de peso, porque atua na regulação dos neuropeptídeos associados diminuindo a

ingestão de alimentos, cuja ação ocorre nas regiões hipotalâmicas do cérebro que controlam o comportamento alimentar, desempenhando um papel importante na manutenção do metabolismo do corpo (Wasim et al., 2016; Guzmán et al., 2019). Sabe-se que indivíduos obesos apresentam níveis elevados de leptina, causados pelo que se chama de resistência à leptina, semelhante ao que acontece com a resistência à insulina, por exemplo. A resistência à leptina, é uma condição em que a leptina está disponível, mas não reduz a ingestão de alimentos e o acúmulo de gordura. (Ahima et al., 1996; Enriori et al., 2006; Tanakun et al., 2014).

Diversos estudos têm demonstrado associação entre os níveis de leptina e o IMC, podendo esses níveis variarem de acordo com a variação do IMC (Lubkowska et al., 2015; Facey et al., 2017). Neste sentido, Pîrsean et al. (2019) observaram que o IMC foi o principal indicador dos níveis de leptina, pois os valores em crianças com obesidade foram três vezes maiores em comparação aos outros estados nutricionais.

Ao contrário da leptina, a grelina estimula a ingestão de alimentos e aumenta o IMC, promovendo ganho de peso corporal e adiposidade, enquanto diminui o gasto energético e a aumenta gordura corporal (Miljković et al., 2017). Também está associada ao aumento da secreção do hormônio do crescimento, o GH (Nakazato et al., 2001). A grelina tem papel importante na sinalização dos centros hipotalâmicos que regulam a ingestão alimentar e o balanço energético, está envolvido no estímulo para iniciar uma refeição e seus níveis oscilam de acordo com o estado nutricional do indivíduo. Está relacionada a curto prazo com a regulação do balanço energético. Ela é produzida, principalmente no trato gastrointestinal e é também sintetizada em menores quantidades por muitos outros tecidos centrais e periféricos, incluindo o hipotálamo, glândula pituitária, epitélio intestinal, pâncreas, fígado e tecido adiposo (Ibrahim, 2015).

Em suma, a leptina e a grelina são hormônios complementares e antagonistas, enquanto a leptina inibe a grelina estimula o apetite, elas desempenham papel fundamental na homeostase corporal e quando essa homeostase é prejudicada, esses hormônios passam a se desregular.

Li et al. (2011) encontraram correlação positiva significativa entre níveis de grelina plasmática e grelina salivar, que por sua vez foram correlacionadas negativamente com o IMC em crianças e adolescentes obesos, sugerindo que a mensuração de grelina na saliva pode ser um método alternativo eficaz de quantificação associada ao desenvolvimento da obesidade nesta população. Sabe-se que esse hormônio pode estar em menor quantidade na obesidade, porém indivíduos obesos são mais sensíveis à grelina (Makris et al., 2017). Como os níveis de

leptina e grelina podem estar alterados na obesidade, a avaliação destes hormônios é importante para o estabelecimento de novas estratégias diagnósticas e, possivelmente, terapêuticas (Klok et al., 2007).

O índice de massa corporal (IMC) tem sido o método antropométrico mais utilizado para avaliação do estado nutricional, por ser uma medida simples e de baixo custo com adequada concordância entre os indicadores de adiposidade no diagnóstico de sobrepeso e obesidade em crianças (Giugliano e Melo, 2004). Nesta população, em função das alterações de peso e altura durante o crescimento e desenvolvimento, recomenda-se a utilização de referenciais específicos para a idade e gênero, como o diagnóstico de sobrepeso e obesidade proposto pela Organização Mundial da Saúde que se baseia na distribuição do percentil/escore-Z de peso para altura, sendo a relação entre o peso encontrado e o peso ideal para a altura (WHO, 1995).

Diante dos fatores ambientais e comportamentais diversos questionários foram desenvolvidos para avaliar o comportamento alimentar de bebês e crianças, podendo assim, nortear as possíveis causas do aumento da obesidade infantil. O apetite, o prazer em comer, a sensibilidade a fatores externos associados aos alimentos (estímulos sensoriais como o gosto e o aroma, por exemplo) e os aspectos emocionais são considerados determinantes do estilo alimentar relacionados ao incremento da prevalência da obesidade em crianças (Flodmark et al., 2004).

Instrumentos psicométricos têm sido utilizados para avaliar comportamentos alimentares em crianças e adultos para predição de risco de distúrbios alimentares e problemas relacionados ao peso corporal (Gallant et al., 2010). Escores obtidos de questionários respondidos pelos pais ou responsáveis são confiáveis, pois estes estão próximos às crianças em praticamente todas as situações, fornecendo assim informações sobre várias situações cotidianas (Livingstone e Robson, 2000). Webber et al. (2009) verificaram que os escores de instrumentos psicométricos para avaliação de comportamento alimentar mostraram associação com obesidade na infância. A detecção precoce de traços de comportamento alimentar que promovem a obesidade ajudam a identificar grupos de risco e introduzir intervenções. Essa detecção geralmente é feita usando questionários preenchido pelos pais. Esses formulários descrevem o comportamento alimentar das crianças e o relacionam com probabilidade de obesidade (Al-Hamad et al., 2021).

O *Children's Eating Behavior Questionnaire* (CEBQ) tem sido considerado um instrumento confiável para avaliação do comportamento alimentar em crianças. Este

questionário foi desenvolvido e validado na Inglaterra (Wardle et al., 2001) e também traduzido em outros países da Europa, como a Holanda (Sleddens et al., 2008) e Portugal (Viana e Sinde, 2008). O CEBQ foi criado tendo como suporte teórico o conhecimento atual sobre as causas alimentares da obesidade privilegiando, entre estas, os determinantes comportamentais (Viana e Sinde, 2008).

O CEBQ (Wardle et al., 2001) é um questionário desenvolvido especificamente para investigar o comportamento alimentar em crianças e jovens, por meio das respostas fornecidas pelos seus cuidadores. O instrumento é composto por 35 itens e tem por objetivo avaliar oito dimensões. Já o *Baby Eating Behaviour Questionnaire* (BEBQ) foi desenvolvido como um instrumento psicométrico para mensurar o apetite da criança durante o período de amamentação (Llewellyn et al., 2011). Os construtos e os itens do BEBQ foram derivados do CEBQ, suplementado pela literatura relacionada aos comportamentos na amamentação. Crianças com alto risco de obesidade apresentaram padrão de sucção mais ávido (Stunkard et al., 2004). Um estilo vigoroso de amamentação na segunda a quarta semana de vida, caracterizado por alta pressão de sucção com períodos de repouso curtos, foi associado à alta adiposidade dois anos após (Agras et al., 1990). Li R et al. (2008) demonstraram que o uso de mamadeira nos seis primeiros de vida foi preditor de excesso de peso durante o segundo semestre de vida. Sendo assim, variação nas características do apetite associada com a susceptibilidade à obesidade pode ser determinada nas primeiras semanas de vida e estão intimamente ligadas ao aleitamento materno.

Neste contexto, este estudo teve por objetivo investigar longitudinalmente o comportamento alimentar, o tempo de aleitamento materno exclusivo, a dosagem dos biomarcadores leptina e grelina salivar e associá-los com o sobrepeso e a obesidade aferidos através do percentil/score Z em bebês a partir dos 5 meses de idade, acompanhados durante 3 anos.

2 ARTIGO: OVERWEIGHT AND OBESITY, FOOD BEHAVIOR AND SALIVARY APPETITE BIOMARKERS IN EARLY CHILDHOOD. A LONGITUDINAL STUDY

Artigo submetido ao periódico: Journal of Pediatric Nursing

Gabriela Novo Borghi ¹ Samuel de Carvalho Chaves Junior ² Maria Beatriz Duarte Gavião

¹DDS, MS, PhD Student Departament of Health Science and Pediatric Dentistry – Piracicaba Dental School – University of Campinas – Piracicaba – São Paulo – Brazil - Phone: +55 (19) 993829292. E-mail: gabinovoborghi@gmail.com

²DDS, MS, PhD Student Departament of Health Science and Pediatric Dentistry – Piracicaba Dental School – University of Campinas – Piracicaba – São Paulo – Brazil - Phone: +55(19)983724294 .E-mail: sam_chavesjr@yahoo.com.br

³DDS, PhD, Full Professor, Departament of Health Science and Pediatric Dentistry – Piracicaba Dental School – University of Campinas – Piracicaba – São Paulo – Brazil - Phone: +55(19)997269128.E-mail: mbgaviao@unicamp.br

Corresponding address:

Profa. Maria Beatriz Duarte Gavião - Departament of Health Science and Pediatric Dentistry – Piracicaba Dental School – University of Campinas – Piracicaba – São Paulo – Brazil
Av. Limeira,275–Areião-13414903, Piracicaba, SP, Brasil
e-mail:mbgaviao@unicamp.br

Article submitted to the Journal of Pediatric Nursing:

ABSTRACT

Purpose: To evaluate eating behavior, breastfeeding, and anthropometric characteristics regarding nutritional status from the first months of life, one, and two years after. Salivary ghrelin and leptin were quantified.

Methods: Children aged 5-17 months (n=122) were initially selected (1st phase) and followed up after one (n=100) (2nd phase) and two (n=92) years (3rd phase), evaluating anthropometry and nutritional status. Eating behavior was assessed using the Baby Eating Behavior Questionnaire (BEBQ) in the 1st phase and Children's Eating Behavior Questionnaire (CEBQ) in the 3rd phase when saliva was collected.

Results: In the 1st, 2nd, and 3rd phases, 39.4%, 41%, and 30% of the children, respectively, presented overweight/obesity. Children who were breastfed ≥ 6 months remained eutrophic over time. Scores of 'Slowness in eating' subscale (SE, BEBQ) were lower for eutrophic children. CEBQ subscales were similar between different nutritional status. Significant correlations occurred between BEBQ and CEBQ subscales. Salivary leptin values did not differ between eutrophic and overweight/obese children, whereas lower ghrelin values were observed in overweight/obesity. Ghrelin was negatively correlated with percentile and nutritional status, and both with 'Satiety Responsiveness' (CEBQ). Leptin positively correlated with SE (CEBQ) and negatively with birth weight.

Conclusion: As children breastfed ≥ 6 months remained eutrophic over time, the protective effect of breastfeeding may be suggested. Eating behavior was similar for different nutritional status, except for SE (BEBQ), but the frequency of overweight/obesity suggests early unhealthy eating. Leptin values were similar between nutritional status, while lower ghrelin values were related to higher percentiles and nutritional status.

Keywords - Breastfeeding, eating behavior, childhood obesity, ghrelin, leptin

INTRODUCTION

Obesity is defined as a chronic degenerative pathology, resulting from the accumulation of adipose tissue in an accentuated way, called a multifactorial disease, that is, it happens through a complex interaction between factors such as behavioral, genetic, physiological and psychological (Nascimento et al., 2016).

Childhood obesity is considered a global public health problem and according to the World Health Organization (WHO, 2021) more than 38.9 million children under 5 years of age are overweight. In recent years it was possible to observe an increase in the prevalence of obesity in general, in all age groups (OMS, 2021). The increase in childhood obesity in Brazil is worrying, and it is estimated that 6.4 million children are overweight in Brazil and 3.1 million have already progressed to obesity.

Cunha et al. (2021) highlighted that breastfeeding is included in a period of great importance in child metabolic development, called the “first thousand days” and could affect the development of overweight and obesity (Pietrobelli et al., 2017). Thus, breastfeeding is inversely associated with childhood overweight and obesity, suggesting a possible protective effect (Ardic et al., 2019; Qiao, Jia et al., 2020). In addition, several hypotheses have been raised to explain why breastfeeding protects children against obesity, some refer to the protective effect that would be involved in the specific and unique composition of human milk and also to environmental and behavioral factors (Pauli, 2013). Furthermore, breast milk has bioactive substances in its composition, such as ghrelin and leptin, which may have a protective effect against future obesity, due to its regulatory action on appetite and satiety (Qiao et al., 2020; Brazilian Society of Pediatrics, 2018). The increase in ghrelin levels and the decrease in leptin levels alter the pattern of food intake, increasing the feeling of hunger and decreasing satiety, respectively, resulting in nutritional maladjustments in children (Arslan et al., 2010).

Children with overweight or obesity have been reported to have higher food intake when exposed to food, exhibiting faster food intake, as well as less sensitivity to signs of satiety when compared to their healthy weight counterparts (Llewellyn *et al.*, 2011; Quah *et al.*, 2015). Thus, early detection of eating behavior traits helps to identify risk groups of overweight and obesity and introduce interventions. This detection is usually done using questionnaires completed by parents (Al-Hamad et al., 2021), such as the Children's Eating Behavior Questionnaire (CEBQ) (Wardle et al., 2001) and the Baby Eating Behavior Questionnaire (BEBQ) (Llewellyn et al., 2011).

The CEBQ has been considered one of the most reliable instruments for assessing eating behavior in children, through the answers provided by their caregivers. It was developed by Wardle et al. (2001) with good internal and external reliability and validated by Carnell and Wardle (2008). The CEBQ was created having as theoretical support the knowledge about the dietary causes of obesity, privileging, among these, the behavioral determinants (Viana and Sinde, 2008). The BEBQ was developed as a psychometric instrument to measure the child's appetite during the breastfeeding period (Llewellyn et al., 2011). Its constructs and items were derived from the CEBQ, supplemented by literature related to breastfeeding behaviors. Thus, variation in appetite characteristics associated with obesity susceptibility can be determined in the first weeks of life and are closely linked to breastfeeding.

In this context, this study aimed to investigate longitudinally the appetite during the period of exclusive milk feeding period in children from 5 months of age and to verify the possible association with the anthropometric measures and nutritional status during two consecutive years, taking in account the period of exclusive breastfeeding. Moreover, feeding behavior in the last evaluation and dosage of salivary leptin and ghrelin biomarkers were assessed.

MATERIAL AND METHODS

This is a longitudinal, observational study, approved by the Research Ethics Committee of the Piracicaba Dental School, University of Campinas (FOP-UNICAMP), process number 55088216.9.0000.5418. Parents or guardians of children enrolled in day care centers in the Municipality of Piracicaba, São Paulo - Brazil, were informed about the research, the risks and discomforts and possible benefits, and those who agreed with the research signed the free and informed consent form, authorizing the participation of babies.

Sample

A convenience sample composed of 142 parent/guardian-child dyads, with children aged from 5 months, was initially selected. Parents/guardians were contacted and invited to participate in the research in public day care centers chosen by the Department of Education of the municipality of Piracicaba, where the evaluations were carried out. They were followed up and evaluated at three times, from 2017 to 2019: (1) first phase; (2) second phase after one year; (3) third phase after two years of the first evaluation.

Inclusion and exclusion criteria

As inclusion criteria, the children should be enrolled in day care centers in the municipality of Piracicaba, be aged at least 5 months and have the consent of their parents/guardians to participate in the research.

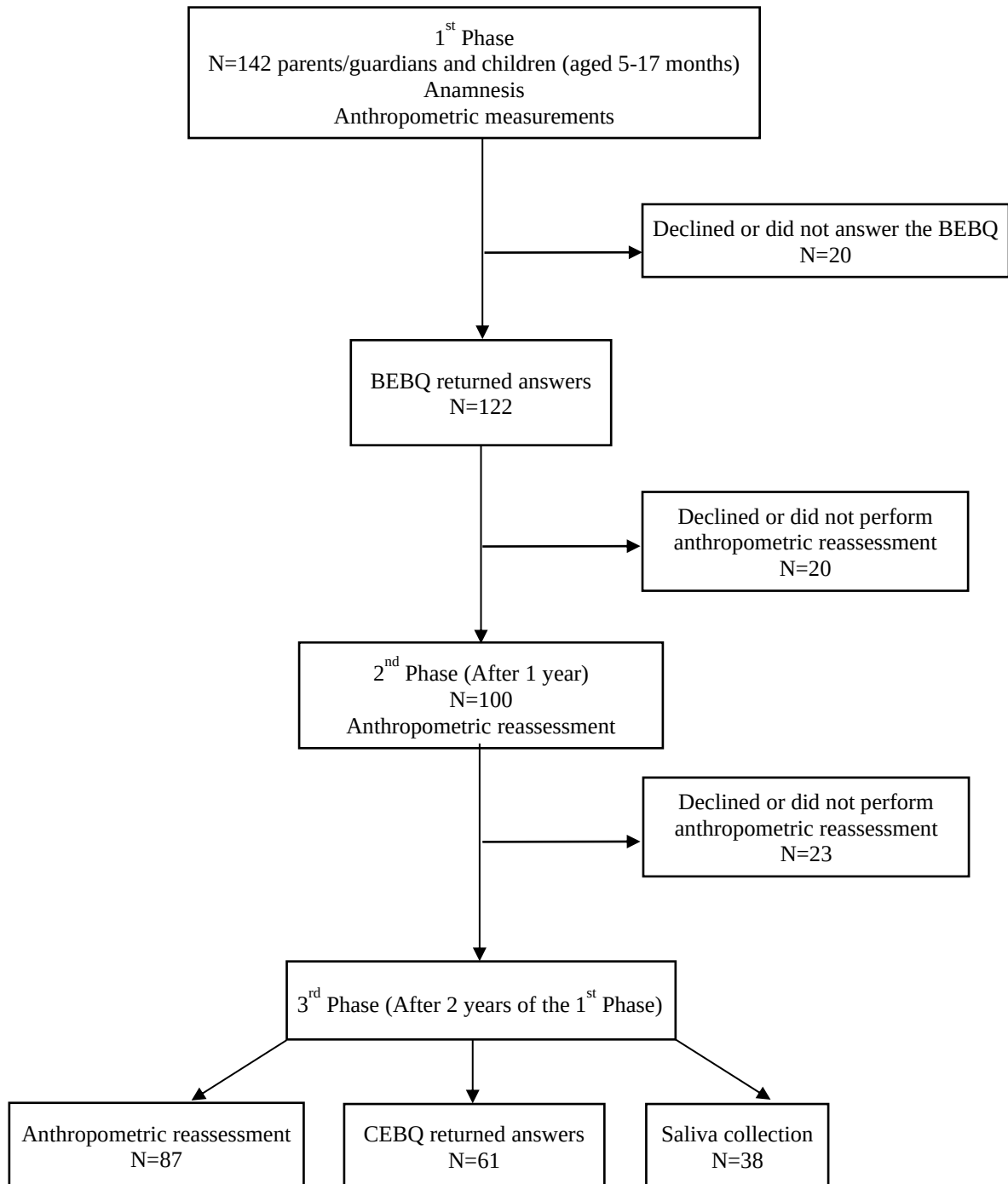
The exclusion criteria considered children who had syndromes or chronic systemic diseases; disorders of systemic origin that could compromise the masticatory system, such as neurological disorders, cerebral palsy, among others; children who did not cooperate with the examination and who refused to participate in the research.

Experimental design

The evaluations were performed by a single previously trained examiner (G.N.B), who received the collaboration of an assistant.

The three phases of the study are described in Figure 1.

Figure 1 – Flowchart of experimental design



Anamnesis

The anamnesis was performed using a pre-structured questionnaire given to the parents/ guardians, verifying prenatal, natal and postnatal medical history. At that moment, items that characterized inclusion and exclusion criteria were searched, according to information provided by those responsible. The following information was obtained:

- Individual biological characteristics: sex; whether premature or not (age at birth <37 weeks), type of delivery, birth weight, health problems during pregnancy;
- Socioeconomic factors: mother's and father's education, family income in monthly minimum wages;
- Factors linked to the mother: mother's age and weight;
- Past nutritional factors: duration of breastfeeding.

Anthropometric assessment

The nutritional status of the children was assessed based on anthropometric data, which were assessed in the three phases. The measurement of weight and height was performed according to standardized norms of the World Health Organization (Onis et al., 2007). Weight was measured using a scale and height using a portable stadiometer, according to the child's age, at the respective assessment times. The calculation of Body Mass Index (BMI) and height for age, in the form of Z-score and percentile, was performed using the WHO Anthro Plus statistical package (WHO, 2009). The anthropometric reference adopted was that of the World Health Organization (Onis et al., 2007), which considers changes in weight and height, adopting the following cut-off points:

- < 0.1 percentile (< z score -3) - Pronounced thinness
- > 0.1 percentile and < 3 percentile (> z score -3 and < z score -2) - Thinness
- > 3rd percentile and < 85th percentile (> z score -2 and < z score +1) - Eutrophy
- > 85th percentile and < 97th percentile (> z score +1 and < z score +2) - Overweight
- > 97th percentile and < 99.9 percentile (> z score +2 and < z score +3) – Obesity

Appetite traits during the milk feeding period

Appetitive traits were measured using the Baby Eating Behavior Questionnaire (BEBQ), which is a self-administered questionnaire on eating behavior, measuring four aspects of appetite that may influence weight and the appetite in general (Llewellyn et al., 2011). For the present study, the Brazilian version was applied (Lucion et al., 2017). It consists of 18 multiple-choice questions with four subscales to describe different food characteristics and one more question that describes appetite in general (Chart 1) (Llewellyn et al., 2011):

Chart 1 – Definition and division of BEBQ subscales.

BEBQ subscales	Definition
(1) Food Responsiveness (6 questions)	They represent greater interest in food and greater response to external signals related to them.
(2) Enjoyment of food (4 questions)	
(3) Satiety Responsiveness (3 questions)	It reflects sensitivity to internal signs of satiety and, therefore, appetite regulation ability and greater effectiveness in controlling caloric intake, constituting some protection against excessive food consumption.
(4) Slowness in Eating (4 questions)	It reflects a lack of pleasure and interest in food and is associated with low body weight in children.
(5) General appetite (1 question)	General disposition toward food.

Responses are given on a five-point Likert scale, where: 1 = never, 2 = rarely, 3 = sometimes, 4 = often and 5 = always.

The mean scores must be calculated for each subscale, with a higher score indicating greater subscale characteristic.

The questionnaire was given to parents/guardians, along with each child's school notebook and returned in the same way. They were asked to complete the BEBQ retrospectively for the period when children were still exclusively milk fed. After reversal of appropriate items, the item scores of each subscale must be averaged to obtain a continuous score for each eating behavior (two items were reversed: one in Slowness in eating and one in Enjoyment of food).

Eating behavior traits

At the third stage, the parents/guardians were asked to fill the Children Eating Behavior Questionnaire (CEBQ), which consists of 35-item parent-report measures assessing eight eating behavior traits. It is conceptually organized into 8 subscales (Chart 2) (Wardle et al., 2001). The European Portuguese version of CEBQ was used (Viana and Sinde, 2008), after being semantically adapted to Brazilian Portuguese language (Silva and Gavião, 2017).

Chart 2 - Definition and division of CEBQ subscales

CEBQ subscales	Definition
(1) Enjoyment of food (EF) (4 questions)	It represents a general interest in food, captures the extent to which the child finds pleasure in eating and wants to eat.
(2) Food Responsiveness (FR) (5 questions)	Measures food intake in response to external food stimuli. It represents a manifest interest in eating and a desire to spend/dedicate time to eating.
(3) Emotional Overeating (EOE) (4 questions)	It represents an increased food intake in response to negative emotions such as anger and anxiety.
(4) Desire to Drink (DD) (3 questions)	It aims to identify the desire to drink, particularly sugary drinks.
(5) Food Fussiness (FF) (6 questions)	It reflects a lack of interest in food and in trying new foods (neophobia), resulting in a poor diet.
(6) Emotional Undereating (EUE) (4 questions)	Represents a decreased food intake for food. It captures the extent to which the child finds pleasure in eating and wants to eat.
(7) Slowness in Eating (SE) (4 questions)	Measures the speed of food intake during a meal. A higher score on this subdimension reflects a gradually reduced interest in the meal.
(8) Satiety Responsiveness (SR) (5 questions)	Represents the degree of self-regulation of the amount of food consumed by a child, based on the child's own feeling of satiety.

The first four subscales comprise “food approach” traits that characterize a larger, more avid appetite and a greater interest in food. Higher scores on these scales indicate a heartier appetite. The last four comprise “food avoidant” traits that characterize a smaller appetite and lower interest in food. Higher scores on these scales indicate a smaller appetite.

The answers are given on a 5-point Likert scale (in which the answer “never” is scored as 1 and “always” as 5).

The questionnaire was handed over to the guardians, along with the child's school notebook and returned in the same way.

After reversal of appropriate items, the item scores of each subscale must be averaged to obtain a continuous score for each eating behavior (five items were reversed: one in Satiety responsiveness, one in Slowness in eating and three in Food Fussiness).

Saliva collection – salivary biomarkers of appetite- ghrelin and leptin

Saliva collection was performed in the third phase of the study, within the day care centers. A subsample composed of 38 children was selected, divided into two groups, one with eutrophic children (n = 21) and the other with overweight/obesity children (n = 17).

Saliva collection was managed by the researcher on the day of the clinical examination. Stimulated saliva was collected using coded salivettes (Salivette®, Sarstedt, Germany) containing a cotton roll, which was placed on the tongue, held, and moved in the child's mouth for one to two minutes, until it was soaked with saliva. Then, the roll was inserted into the salivette and immediately stored on ice to be transported to the laboratory. Saliva collections were performed in the morning or afternoon, with an interval of one hour between the last meal and collection. The child could not have brushed his teeth before collection. In the laboratory, the salivettes were centrifuged at 3000 rpm for 15 minutes and the waste was discarded, only the supernatant was used in 2 microcentrifuge tubes, one for leptin and the other for ghrelin. Samples were stored at -80 °C until biomarker quantification was performed. For biomarker dosages, samples were dosed in duplicates, so that samples from the same individual were dosed in the same test. On the test day, the samples were thawed in a refrigerator and centrifuged again at 3,000 rpm for 10 minutes. Saliva was measured with the Elisa, Human LEP (Leptin) ELISA Kit and Human GHR (Ghrelin) ELISA Kit (Elabscience®) kits, according to the manufacturer's instructions. Results were expressed in pg/ml.

Data analysis and statistics

Study data were analyzed using descriptive statistics. Normality was verified to direct parametric or non-parametric tests for comparisons and correlations with Shapiro-Will test. The scores of the subscale of BEBQ and CEBQ were averaged, obtaining a continuous score for each subscale. For comparisons Student's t test or Mann-Whitney were applied. Proportions were compared using Fisher's Exact and Chi-square tests, when indicated. To

correlate the variables, the Spearman or Pearson coefficient was applied according to the data distribution. For some comparisons and associations, data from overweight and obese children were pooled. SPSS software (SPSS Inc., Chicago, IL, USA) was used. The level of significance considered was $\alpha=0.05$.

RESULTS

In Table 1, the general and demographic characteristics of the sample collected in the first phase of the study are demonstrated. The mean age in the first phase was about 12 months. The frequency of boys and girls did not differ significantly. More than 60% of parents declared having attended high school or higher education, a proportion that differs significantly from those without schooling or elementary school. In addition, the family income of 67% of the sample was around 1 to 3 monthly minimum wage.

Table 1- Sociodemographic characteristics of the sample (collected on baseline)

		n	%
Children's age (months) [mean (SD)]	12.60 (2.59)	142	100
Sex	Boys/girls	81/61	57.04/42.95
Age when mother got pregnant [mean (SD)]	28.07 (6.42)	-	-
Birth weight (g) [mean (SD)]	3143.35 (775.31)	-	-
Way of birth	Normal birth	38	26.27
	Cesarean	82	57.75
	No answer	22	15.49
Premature born	Premature	6	4.23
	Full born	114	80.28
	No answer	22	15.49
Race	White	85	59.85
	Not white	57	40.14
Father's education	No schooling	2	1.40
	Fundamental	25	17.60
	Medium	78	54.92
	Higher	15	10.56
	No answer	22	15.49
Mother's education	No schooling	0	0
	Fundamental	17	11.97
	Medium	81	57.04
	Higher	29	20.42
	No answer	15	10.56
Family income [†]	Less than 1 wage	8	6.56
	1 to 2 wages	43	35.25
	> 2 to 3 wages	31	25.41
	> 3 to 4 wages	10	8.2
	> 4 to 5 wages	5	4.1
	> 5 wages	2	1.64
	No answer	23	18.85

[†]Monthly minimum wage in 2017 – R\$ 937.00; 1 US\$ = R\$ 3.31

Table 2 shows the anthropometric data and the distribution of children according to the weight, height, BMI, percentile, nutritional status, and breastfeeding duration in the three phases of the study. Weight and height increased significantly, as expected. BMI decreased through the three phases, whereas percentiles did not show significant differences. Pronounced thinness and thinness presented low frequency, while eutrophic was the most frequent nutritional status in the three phases. More than a third of the sample was overweight and obese throughout the three phases. The proportion of children in each nutritional status did not differ significantly between the respective study phases. The duration of breastfeeding was categorized as “not breastfed”, “breastfed less than six months”, and breastfed for six months or more. The proportion of children who were breastfed for six months or more were significantly higher in the three phases in relation those not breastfed or breastfed less than six months. The distribution and respective associations between nutritional status according to the duration of breastfeeding in each study phase can be seen in Supplementary Table 1.

Table 2. Descriptive anthropometric data and nutritional status of children throughout the three study phases

		Phase 1 (n=122)	Phase 2 (n=100)	Phase 3 (n=92)	P-values
Weight (kg)	Mean (SD)	10.0 (1.43) ^A	13.7 (1.92) ^B	15.7 (2.51) ^C	Kruskal-Wallis P<0.0001 Dunn P<0.05
	Median (25%-75%)	9.97 (9.16 - 10.8)	13.80 (12.5 - 14.5)	15.8 (14.1 - 17.3)	
	Range	6.85 - 13.90	9.00 - 19.4	9.30 - 27.1	
Height (cm)	Mean (SD)	75.20 (4.64) ^A	90.3 (3.90) ^B	99.0 (3.51) ^C	Kruskal-Wallis P<0.0001 Dunn P<0.05
	Median (25%; 75%)	75.00 (72; 78)	91.0 (87.0; 93.0)	99.3 (96.4; 102)	
	Range	65.00 - 86.00	82.00 - 101.00	89.0 - 106	
BMI	Mean (SD)	17.70 (1.72) ^A	16.8 (1.58) ^B	16.0 (1.99) ^C	Kruskal-Wallis P<0.0001 Dunn P<0.05
	Median (25%-75%)	17.60 (16.70 - 19.00)	16.7 (16.0 - 17.7)	15.9 (14.9; 17.2)	
	Range	11.90 - 21.4	12.2 - 22.2	10.2 - 24.1	
Percentile	Mean (SD)	69.10 (27.4) ^A	73.2 (25.7) ^A	62.4 (32.4) ^A	Kruskal-Wallis P=0.157
	Median (25%-75%)	72.80 (51.10; 92.80)	81.8 (60.0; 93.1)	70.3 (0.9; 1.02)	
	Range	0.10 - 99.90	0.20 - 99.9	0.89 - 1.06	
Nutritional status [n (%)]	Pronounced thinness	1 (0.8 %)	-	2 (2.2 %)	Inter-phases χ^2 Test; P= 0.678
	Thinness	2 (1.6 %)	3 (30.0 %)	3 (3.3 %)	
	Eutrophic	71 (58.2 %)	56 (59.0 %)	59 (64.1 %)	Intra-phase Fisher's exact test; P<0.0001
	Overweight	29 (23.8 %)	26 (26.0 %)	17 (18.5 %)	
	Obesity	19 (15.6 %)	15 (15.0 %)	11 (12.0 %)	
Breastfeeding [n (%)]	Not breastfed	16 (14.3%)	11 (12.6%)	9 (16.1%)	Inter-phases χ^2 Test P= 0.972
	< 6 months	34 (30.4%)	29 (33.3%)	18 (32.1%)	
	≥ 6 months	62 (55.4%)	47 (54.0%)	29 (51.8%)	Intra-phase χ^2 Test; P<0.01

Different superscript capital letters mean significant differences between phases

The values of BEBQ subscales were compared between eutrophic children and those presenting overweight/obesity (Table 3). Given that the distributions of those values were not normally distributed, median, intervals, and ranges were also presented. Pronounced thin and thin children were excluded from the comparison analysis because of the low frequency. ‘Slowness in eating’ was the only subscale that differed between eutrophic and overweight/obese children, in line with the subscale definition. The other subscales were similar between the two nutritional status.

Table 3. Descriptive data of BEBQ subscales according to nutritional status and the respective comparisons

BEBQ subscales		Eutrophic n = 71	Overweight/Obesity n = 48	P-value
Food Responsiveness	Mean (SD)	2.56 (0.92)	2.53 (0.95)	0.797 ^a
	Median (25-75%)	2.5 (1.83-3.17)	2.33 (1.83 - 3.04)	
	Range	1 - 5	1.17 - 4.67	
Enjoyment of food	Mean (SD)	4.50 (0.67)	4.59 (0.45)	0.916 ^a
	Median (25-75%)	4.75 (4.25 - 5)	4.75 (4.25 - 5)	
	Range	1.25 - 5	3.25 - 5	
Satiety Responsiveness	Mean (SD)	2.41 (0.70)	2.54 (0.76)	0.429 ^a
	Median (25-75%)	2.33 (2 - 3)	2.33 (1.67 - 2.67)	
	Range	1 - 4	1.33 - 4.33	
Slowness in Eating	Mean (SD)	2.69 (0.70)	2.43 (0.68)	0.043 ^b
	Median (25-75%)	2.75 (2.25 - 3)	2.38 (2 - 3)	
	Range	1 - 5	1 - 4	
General appetite	Mean (SD)	4.31 (0.97)	4.46 (0.80)	0.579 ^a
	Median (25-75%)	5 (4 - 5)	5 (4-5)	
	Range	1 - 5	2 - 5	

^a Mann-Whitney test

^b Student's test

In the third phase all parents/guardians of 87 examined children were asked to answer the CEBQ, and 61 questionnaires were returned. Comparison of CEBQ scores were between eutrophic children and those presenting overweight/obesity are in Table 4. The subscale values were similar between the nutritional status.

Table 4. Mean scores of CEBQ subscales according to nutritional status

		Eutrophic N=39	Overweight/obesity N=18	P-values
Enjoyment of Food	Mean (SD)	3.54 (0.79)	3.90 (0.63)	0.090 ^a
	Median (25; 75%)	3.5 (3; 4)	4 (3.56; 4.25)	
	Range	2 - 5	2.75 - 5	
Food Responsiveness	Mean (SD)	2.34 (0.77)	2.76 (0.86)	0.082 ^b
	Median (25; 75%)	2.2 (1.8; 2.8)	2.7 (2.05; 3)	
	Range	1 - 4.8	1.6 - 5	
Emotional Overeating	Mean (SD)	2.01 (0.93)	2.07 (0.97)	0.802 ^b
	Median (25; 75%)	2 (1.25; 2.5)	2 (1.25; 2.5)	
	Range	1 - 4.75	1 - 4.5	
Desire to Drink	Mean (SD)	3.12 (1.04)	2.67 (1.17)	0.146 ^a
	Median (25; 75%)	3 (2.33; 4)	2.83 (1.67; 3.33)	
	Range	1 - 5	1 - 5	
Food Fussiness	Mean (SD)	3.11 (0.48)	3.09 (0.51)	0.936 ^a
	Median (25; 75%)	3.17 (2.83; 3.42)	3 (2.83; 3.29)	
	Range	2.17 - 4.17	2.33 - 4.2	
Emotional Undereating	Mean (SD)	2.84 (1.06)	2.51 (1.03)	0.283 ^a
	Median (25; 75%)	3 (1.88; 3.5)	2.25 (2; 3)	
	Range	1 - 5	1 - 5	
Slowness in Eating	Mean (SD)	3.21 (0.69)	3.07 (0.66)	0.464 ^a
	Median (25; 75%)	3.25 (2.63; 3.75)	3 (2.56; 3.44)	
	Range	1.5 - 4.5	1.75 - 4.25	
Satiety Responsiveness	Mean (SD)	2.99 (0.54)	2.92 (0.51)	0.654 ^a
	Median (25; 75%)	3 (2.6; 3.4)	3 (2.6; 3.15)	
	Range	1.8 - 4	2 - 4.2	

^a Student's t test ^b Mann-Whitney test

Table 5 shows the comparison analysis of the dosages of the salivary leptin and ghrelin in the subsample, according to the weight status, in third phase. Overweight/obese children had significantly lower ghrelin values, whereas for leptin, no significant difference between groups occurred.

Table 5 – Comparison of the Salivary Leptin and Ghrelin levels according to the nutritional status in the third phase of the study

		Eutrophic N=21	Overweight/obesity N=17	P-value*
Leptin (pg/ml)	Mean (SD)	0.18 (0.23)	0.11 (0.08)	0.547
	Median (25;75 %)	0.09 (0.07; 0.12)	0.08 (0.07; 0.11)	
	Range	0.06-1.01	0.06-0.38	
Ghrelin (pg/ml)	Mean (SD)	0.71 (0.08)	0.60 (0.18)	0.006
	Median (25;75 %)	0.70 (0.68; 0.74)	0.66 (0.59; 0.70)	
	Range	0.45-0.90	0.12-0.87	

* Mann-Whitney U

Correlation between variables was performed for each phase of the study. In phase 1 subscales of BEBQ, percentiles, nutritional status, and breastfeeding duration were correlated. In the second phase the current anthropometric measurements and nutritional status, BEBQ subscales obtained in the first phase, and breastfeeding duration were correlated. Correlations between variables in the third phase of the study were carried out, as done in the other two phases, adding CEBQ and leptin and ghrelin values. Table 6 shows only the relevant and significant correlations between variables in each phase of the study. Correlation matrices with all variables for each phase can be accessed in Supplementary Tables 2, 3, and 4.

In the first phase, breastfeeding was positively correlated with ‘food responsiveness’ and ‘slowness in eating’ and ‘general appetite’. The correlations between ‘slowness in eating’ with percentile and nutritional status were negative, as expected, since interest in food may be diminished, meaning influence on low body weight.

In the second phase, ‘slowness in eating’ was negatively correlated with percentile maintaining the same pattern found in first phase, that is, lesser interest in food expressed by higher

scores in this subscale was inverse to body measurements. Breastfeeding was negatively correlated with nutritional status.

Significant correlations occurred between BEBQ and CEBQ subscales, showing that appetite during milk feeding period tended to be like eating behavior traits after two years. Exception was seen between Food Responsiveness (CEBQ) and Enjoyment of Food (BEBQ), which were negatively correlated. Current percentile and nutritional status showed inverse relation with Satiety Responsiveness in CEBQ and in BEBQ, respectively. Moreover, ghrelin values were negatively correlated with percentile and nutritional status which agrees with the results of comparison between nutritional status and ghrelin values, that in turn were positively correlated with the Food Enjoyment (BEBQ). On the other hand, leptin was negatively correlated with slowness in eating (CEBQ).

Table 6 - Correlations between variables during the three phases of the study

			Spearman's rho	P-value	N
First Phase	Food Responsiveness (BEBQ)	Breastfeeding ¹	0.26	0.006	115
		Percentile	-0.23	0.012	121
	Slowness in eating (BEBQ)	Nutritional status	-0.19	0.040	122
		Breastfeeding ¹	0.22	0.019	115
	General appetite (BEBQ)	Breastfeeding ¹	0.19	0.044	115
Second Phase	Slowness in eating (BEBQ)	Percentile	- 0.23*	0.030	90
	Breastfeeding ¹	Nutritional status	- 0.23*	0.032	90
Third Phase	Food Responsiveness (CEBQ)	Enjoyment of Food (BEBQ)	-0.31	0.016	60
		Food Responsiveness (BEBQ)	0.29	0.024	60
	Desire to Drink (CEBQ)	Slowness in Eating (BEBQ)	0.29	0.025	60
	Satiety Responsiveness (CEBQ)	Percentile	-0.31	0.022	55
	Satiety Responsiveness (BEBQ)	Nutritional status	-0.29	0.027	59
	Leptin	Slowness in Eating (CEBQ)	-0.37	0.024	38
	Ghrelin	Percentile	-0.41	0.013	37
		Nutritional status	-0.43	0.007	38

¹Breastfeeding (ordinal: not, < 6 months, ≥ 6 months)

* Pearson's r

It must be considered that all correlation coefficients were low, meaning weak correlations. Therefore, the respective results should be interpreted with caution. In addition, there was a loss of the sample between the three evaluations, due to the withdrawal of those responsible, the lack of adherence to filling out the questionnaires and the expected loss of volunteers during the years of evaluations.

DISCUSSION

Given the scenario in which there is an increase in the prevalence of overweight and obesity in children in early childhood, conditions that are currently considered a public health problem, the present study is justified. The early detection of parameters involved, such as exclusive breastfeeding, characteristics of eating behavior and salivary biomarkers of appetite become important to support preventive strategies, although obesity is a complex condition with several factors involved.

The sample consisted of children of both sexes from five months of age. The proportion of boys and girls was similar, and their parents had a good level of education, which ensured sample homogeneity and reliability in the assessments in which the respective participation was necessary.

During the study period, it was found that the frequency of children with normal weight status was significantly higher. However, overweight and obesity were present in more than a third of the sample in the three evaluation phases. Such results are like those reported by ABESO for children aged 5 to 9 years. The aforementioned percentage found in the present study at younger ages is worrying, as it infers those comorbidities related to overweight and obesity can manifest early, corroborating the premises of the Ministry of Health in Brazil. The fact that the proportion of different nutritional states does not differ over approximately three years, denotes the need for educational and preventive measures to favor behavioral patterns.

In table 3, we can see the means and standard deviations of the subscales of the BEBQ questionnaire applied in Phase 1. The 'Enjoyment of food' subscale presented significantly higher scores than the others, both for children with normal weight and for those with overweight or obesity. Between both nutritional statuses, scores in the 'Slow in eating' subscale were significantly higher for eutrophic children. The BEBQ aims to expand knowledge about infant feeding behavior, as it assesses aspects of feeding behavior that can influence body

weight (Wardle et al., 2002). It is interesting to note that the Enjoyment of food subscale was similar for eutrophic and children with overweight or obesity, showing satisfaction in eating. On the other hand, the Slowness in Eating subscale showed significantly higher scores for eutrophic children, which infers that the speed in food intake was reduced compared to overweight and obese children and in less interest in the meal.

Regarding the duration of breastfeeding found in our sample, it was possible to observe that there was a significant negative correlation between nutritional status and duration of breastfeeding in the second phase of the study, meaning that longer breastfeeding duration decreases nutritional status. This is in line with what was observed over the three years, as the proportion of overweight and obese children decreased in phase two and remained in phase three. The number of children who were not breastfed was low and most of them had been breastfed for more than six months, which demonstrates the awareness of those responsible for the evaluated children regarding the importance of natural breastfeeding. However, the frequency found of overweight and obesity in the general scope of the study suggests unhealthy eating from the first years of life, in agreement with Saldiva et al. (2014) and Jensen et al. (2015), in addition to other factors implicated in nutritional status. Therefore, these aspects must be addressed early and effectively, focusing on specific characteristics in the first years of life to encourage natural breastfeeding and appropriate eating behavior, thus preventing the development of obesity (Jensen et al., 2015).

Continuing the study of eating behavior, in the third phase of the study, the CEBQ instrument was used, which was developed to investigate eating behavior in children from three years of age and, consequently, the behavioral determinants of obesity (Wardle et al., 2001). Comparisons of other CEBQ subscales among children with different nutritional status did not show significant differences. Perhaps the similar values in the comparison of CEBQ subscales in the present study can be attributed to the number of children in each category, since volunteers were lost throughout the study, which may have significantly affected the lack of difference between the groups studied.

The variables were correlated to the three study phases. In the first phase, enjoyment of food was not correlated with percentiles, despite high values rated by parents/guardians. This finding agrees with Quah et al. (2015), who also observed no association with weight gain from 3 months up to 24 months of age and Patel et al. (2017). In line with this result, enjoyment of food was negatively responsive correlated with satiety, that makes sense, since self-regulation of the amount of food consumed is based on the child's own feeling of satiety. Moreover,

negative correlations between general appetite with food responsiveness and satiety can be considered plausible. Significant correlations between breastfeeding, duration and BEBQ subscales may show the influence of appetite traits on nutritional status. In the second phase percentile maintained negative correlation with slowness in eating as occurred in the first phase, showing that this appetite trait can influence body measurements early. Percentile and nutritional status in the third phase were negatively correlated with Satiety Responsiveness (CEBQ) and Satiety Responsiveness (BEBQ), respectively, showing that food avoidance traits in fact characterize a smaller appetite and lower interest in food.

The leptin levels found in eutrophic and obese children in the present study were similar, agreeing with Pîrsean et al. (2019) who found that despite the salivary leptin level was highly variable in obese children, similar levels were found in control children. They also corroborate the findings of Ibrahim Abdalla in 2018, who found no differences in salivary leptin levels between the groups of eutrophic and obese young male adults. Conversely, Warketin et al. (2020) found that children with overweight/obesity at age 7 years showed higher leptin than the ones with normal weight. The differences between the studies can be attributed to methodologic approaches, such as the sample age and leptin assessment using saliva or plasma concentration. In addition, our sample size was very small, which could be considered a limitation of the present study. On the other hand, an inverse relationship between ‘Slowness in eating’ (CEBQ) and leptin level occurred, agreeing with Warketin et al. (2020). This finding could be attributed to role of leptin on food ‘Satiety responsiveness’ (Liao et al., 2021), since higher scores on ‘Slowness in eating’ and ‘Satiety responsiveness’ subscales indicate a smaller appetite or food avoidant behaviors (Warketin et al., 2020). The other subscales were not correlated with salivary leptin levels, as also found previously in school children (Saet al., 2018). In contrast, leptin levels were positively associated with food approach in Warketin et al. (2020) study. Again, the divergent results may be due the nature of the CEBQ and type of collected leptin. Moreover, CEBQ can be viewed as a descriptive measure of current (acute) appetite-regulation that includes assessments of hunger and fullness whereas leptin is considered a tonic signal based on fat mass and overall energy balance (Cohen et al., 2018).

Corroborating previous studies by Nakazato et al. (2001) and Miljković et al. (2017) that found decreased ghrelin levels due to increased caloric intake in obese patients, our findings showed significantly lower ghrelin values ($p=0.006$) for children with overweight/obesity than for normal weight children. In fact, studies have shown that ghrelin levels are lower in subjects with obesity compared to control, as commented by Pierse et al. (2015). Low plasma ghrelin

levels were also observed by Wali et al. (2014) in children with obesity and when these children have reduced 50% of their BMI, ghrelin levels remained lower in comparison to control subjects. Moreover, in 2002 English et al. have already observed that the decrease in ghrelin after a meal was lower in individuals with obesity compared to eutrophic ones, thus being able to maintain the feeling of hunger. In this context, contrary to what was expected, an inverse relationship between ghrelin levels and the body mass index (BMI) has been demonstrated (Tucci, 2008; Fabbri et al., 2015), as found in the present study. There was a negative correlation ($p=0.013$) between ghrelin levels and the percentile and nutritional status, in agreement with the data found above, suggesting that the increase in ghrelin levels is correlated with eutrophic nutritional status, also corroborating the results of Önnarfält et al. (2018). A literature review (Lewis and Brown, 2017) showed that interventions for weight management in children achieved a decrease in leptin and increase in ghrelin, and recently, Liao et al. (2021) observed that ghrelin was improved by 6-week combined exercise and dietary interventions in 12-year-old children with obesity. However, no relationship was found between salivary ghrelin levels and appetite traits in the present study since the respective correlations with the CEBQ subscales were not significant, corroborating Liao et al. (2021). As commented above, the nature of the CEBQ could be a determinant factor.

Research on eating behavior is essential for the improvement and development of specific measures, guiding clinical and behavioral approaches involved in the attempt to reduce the growing number of overweight and obese children. In addition, knowing the importance of breastfeeding in the first years of the baby's life, it is expected that more research will be carried out to support strategies to protect against obesity.

It was concluded that the most frequent nutritional status in the three phases was eutrophic. Children who were breastfed ≥ 6 months remained eutrophic over time, suggesting a protective effect of breastfeeding. Overweight/obese children had significantly lower salivary ghrelin values than normal weight children, showing that the lower the ghrelin values, the higher the percentile or nutritional status. For leptin, there was no significant difference between groups. The frequency found of overweight and obesity in their general scope of the study suggests unhealthy eating from the first years of life. The subscales of the questionnaires on eating behavior did not show differences between the eutrophic and overweight/obese groups.

REFERENCES

- ABESO – Associação Brasileira para o estudo da obesidade e da síndrome metabólica. Diretrizes brasileiras de obesidade (online). Disponível em: <http://www.abeso.org.br/atitude-saudavel/mapa-obesidade>.
- Al-Hamad AH, Al-Naseeb AM, Al-Assaf MS, Al-Obaid SA, Al-Abdulkarim BS, Olszewski PK. Preliminary Exploration of Obesity-Related Eating Behaviour Patterns in a Sample of Saudi Preschoolers Aged 2-6 Years through the Children's Eating Behaviour Questionnaire. *Nutrients*. 2021 Nov 20;13(11):4156. doi: 10.3390/nu13114156.
- Ardic C, Usta O, Omar E, Yıldız C, Memis E. Effects of infant feeding practices and maternal characteristics on early childhood obesity. *Arch Argent Pediatr*. 2019 Feb 1;117(1):26-33. English, Spanish. doi: 10.5546/aap.2019.eng.26.
- Arslan N, Erdur B, Aydin A. Hormones and cytokines in childhood obesity. *Indian Pediatr*. 2010 Oct;47(10):829-39. doi: 10.1007/s13312-010-0142-y.
- Carnell S, Wardle J. Appetitive traits and child obesity: measurement, origins and implications for intervention. *Proc Nutr Soc*. 2008 Nov;67(4):343-55. doi: 10.1017/S0029665108008641.
- Cohen TR, Hazell TJ, Vanstone CA, Rodd C, Weiler HA. Changes in eating behavior and plasma leptin in children with obesity participating in a family-centered lifestyle intervention. *Appetite*. 2018 Jun 1;125:81-89. doi: 10.1016/j.appet.2018.01.017. Epub 2018 Feb 2. PMID: 29410008.
- Cunha MM, Moraes VT, Gislayne Castro Nieto. Aleitamento materno: qual a sua relação com a obesidade infantil? *Jornal Paranaense de Pediatria* - 2021; 22(1):01-04. Disponível em: <http://www.jornaldepediatria.org.br>.
- English PJ, Ghatei MA, Malik IA, Bloom SR, Wilding JP. Food fails to suppress ghrelin levels in obese humans. *J Clin Endocrinol Metab*. 2002;87:2984.
- Fabbri AD, Deram S, Kerr DS,- Cordás DA. Ghrelin and eating disorders. *Arch. Clin. Psychiatry (São Paulo)* 2015, 42 (2) Mar-Apr <https://doi.org/10.1590/0101-60830000000048>
- Ibrahim Abdalla MM. Ghrelin - Physiological Functions and Regulation. *Eur Endocrinol*. 2015 Aug;11(2):90-95. doi: 10.17925/EE.2015.11.02.90. Epub 2015 Aug 19.

Jensen SM, Ritz C, Ejlerskov KT, Mølgaard C, Michaelsen KF. Infant BMI peak, breastfeeding, and body composition at age 3 y. *Am J Clin Nutr*. 2015 Feb;101(2):319-25. doi: 10.3945/ajcn.114.092957. Epub 2014 Dec 3.

Lewis KA, Brown SA. Searching for evidence of an anti-inflammatory diet in children: a systematic review of randomized controlled trials for pediatric obesity interventions with a focus on leptin, ghrelin, and adiponectin. *Biol Res Nurs*. 2017;19:511–530. <https://doi.org/10.1177/1099800417715734>.

Liao J, Huang J, Wang S, Xiang M, Wang D, Deng H, Yin H, Xu F, Hu M. Effects of exercise and diet intervention on appetite-regulating hormones associated with miRNAs in obese children. *Eat Weight Disord*. 2021 Mar;26(2):457-465. doi: 10.1007/s40519-020-00869-9.

Llewellyn CH, van Jaarsveld CH, Johnson L, Carnell S, Wardle J. Development and factor structure of the Baby Eating Behaviour Questionnaire in the Gemini birth cohort. *Appetite*. 2011 Oct;57(2):388-96. doi: 10.1016/j.appet.2011.05.324.

Lucion MK, Kliemann N, Bernardi RF, Loreto BBL, Farias BL, Reis TM, et al. Tradução e Validação Semântica do “Baby Eating Behaviour Questionnaire” (BEBQ), um Instrumento sobre Comportamento Alimentar de Lactentes. *Publ. UEPG Ci. Biol. Saúde*. 2017;23(2):108–117.

Miljković M, Šaranac L, Bašić J, Ilić M, Djindjić B, Stojiljković M, Kocić G, Cvetanović G, Dimitrijević N. Evaluation of ghrelin and leptin levels in obese, lean and undernourished children. *Vojnosanit Pregl* 2017; 74(10): 963–969.

Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. *Nature*. 2001 Jan 11;409(6817):194-8. doi: 10.1038/35051587.

Nascimento VG, da Silva JP, Ferreira PC, Bertoli CJ, Leone C. Maternal breastfeeding, early introduction of non-breast milk, and excess weight in preschoolers. *Rev Paul Pediatr*. 2016 Dec;34(4):454-459. doi: 10.1016/j.rpped.2016.05.004.

Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ*. 2007 Sep;85(9):660-7.

Önnerfält J, Erlanson-Albertsson C, Montelius C, Thorngren-Jerneck K. Obese children aged 4-6 displayed decreased fasting and postprandial ghrelin levels in response to a test meal. *Acta Paediatr.* 2018 Mar;107(3):523-528. doi: 10.1111/apa.14165.

Pauli, Patricia Seibert. Associação entre aleitamento materno e excesso de peso em crianças e adolescentes, uma revisão de literatura. Rio Grande do Sul, 2013. *Pediatrics.* 2014 Sep;134 Suppl 1:S50-5. doi: 10.1542/peds.2014-0646I.

SBPN, Godfrey KM, Pasupathy D, Levin J, Flynn AC, Hayes L, Briley AL, Bell R, Lawlor DA, Oteng-Ntim E, Nelson SM, Robson SC, Sattar N, Singh C, Wardle J, White SL, Seed PT, Poston L. Infant adiposity following a randomised controlled trial of a behavioural intervention in obese pregnancy. *Int J Obes (Lond).* 2017 Jul;41(7):1018-1026. doi: 10.1038/ijo.2017.44.

Pietrobelli A, Agosti M; MeNu Group. Nutrition in the First 1000 Days: Ten Practices to Minimize Obesity Emerging from Published Science. *Int J Environ Res Public Health.* 2017 Dec 1;14(12):1491. doi: 10.3390/ijerph14121491.

Pîrsean C, Neguț C, Stefan-van Staden RI, Dinu-Pirvu CE, Armean P, Udeanu DI. The salivary levels of leptin and interleukin-6 as potential inflammatory markers in children obesity. *PLoS One.* 2019 Jan 3;14(1):e0210288. doi: 10.1371/journal.pone.0210288.

Qiao J, Dai LJ, Zhang Q, Ouyang YQ. A Meta-Analysis of the Association Between Breastfeeding and Early Childhood Obesity. *J Pediatr Nurs.* 2020 Jul-Aug;53:57-66. doi: 10.1016/j.pedn.2020.04.024.

Quah PL, Chan YH, Aris IM, Pang WW, Toh JY, Tint MT, et al. Prospective associations of appetitive traits at 3 and 12 months of age with body mass index and weight gain in the first 2 years of life. *BMC Pediatr.* 2015 Oct 12;15:153. doi: 10.1186/s12887-015-0467-8.

Saldiva SR, Venancio SI, de Santana AC, da Silva Castro AL, Escuder MM, Giugliani ER. The consumption of unhealthy foods by Brazilian children is influenced by their mother's educational level. *Nutr J.* 2014 Apr 3;13:33. doi: 10.1186/1475-2891-13-33.

Silva GP, Gavião MBD. Comportamento alimentar, condições bucais e aspectos nutricionais em pré-escolares. Trabalho de Conclusão de Curso (graduação) – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba. / Gabriela de Paula Silva. – Piracicaba, SP. 24 p. 2017.

Tucci S. Grelina en regulación del apetito y papel en obesidad y trastornos alimentarios: Abordajes terapéuticos. *Rev Venez Endocrinol Metab.* 2008;6(2):15-23.

Viana V, Sinde S, Saxton JC. Children's Eating Behaviour Questionnaire: associations with BMI in Portuguese children. *Br J Nutr.* 2008 Aug;100(2):445-50. doi: 10.1017/S0007114508894391.

Wali P, King J, He Z, Tonb D, Horvath K. Ghrelin and obestatin levels in children with failure to thrive and obesity. *J Pediatr Gastroenterol Nutr.* 2014;58(3):376-81.

Wardle J, Guthrie CA, Sanderson S, Rapoport L. Development of the Children's Eating Behaviour Questionnaire. *J Child Psychol Psychiatry.* 2001 Oct;42(7):963-70. doi: 10.1111/1469-7610.00792.

Warkentin S, Carnell S, Oliveira A. Leptin at birth and at age 7 in relation to appetitive behaviors at age 7 and age 10. *Horm Behav.* 2020 Nov;126:104842. doi: 10.1016/j.yhbeh.2020.104842.

WHO, 2009 Geneva (<https://www.who.int/tools/growth-reference-datafor-5to19-years>). WHO Child Growth Standards for 0-5 years (www.who.int/childgrowth)

World Health Organization. Obesity: preventing and managing the global epidemic - report of a WHO consultation on obesity. Geneva: WHO; 2021.

Supplementary Table 1 - Correlation matrix between variables for the first phase of the study

		Percentile	Nutritional status	Enjoyment of food	Food Responsiveness	Slowness in Eating	Satiety Responsiveness	General appetite	Breastfeeding months	Age pregnancy	Birth weight	Breastfeeding	ordinal
Nutritional status	Spearman's rho	0.884***	—										
	p-value	< .001	—										
	N	121	—										
Enjoyment of food	Spearman's rho	-0.035	-0.008	—									
	p-value	0.705	0.934	—									
	N	121	122	—									
Food Responsiveness	Spearman's rho	-0.036	-0.085	-0.141	—								
	p-value	0.691	0.352	0.121	—								
	N	121	122	122	—								
Slowness in Eating	Spearman's rho	-0.227*	-0.187*	-0.089	0.102	—							
	p-value	0.012	0.04	0.329	0.265	—							
	N	121	122	122	122	—							
Satiety Responsiveness	Spearman's rho	0.01	-0.037	-0.25**	-0.052	-0.026	—						
	p-value	0.91	0.688	0.005	0.572	0.778	—						
	N	121	122	122	122	122	—						
General appetite	Spearman's rho	-0.038	0.019	0.155	0.299***	0.116	-0.209*	—					
	p-value	0.681	0.836	0.089	< .001	0.203	0.021	—					
	N	121	122	122	122	122	122	—					
Breastfeeding months	Spearman's rho	-0.124	-0.223*	0.077	0.19*	0.141	0.125	0.092	—				
	p-value	0.187	0.017	0.414	0.042	0.134	0.184	0.328	—				
	N	114	115	115	115	115	115	115	—				
Age pregnancy	Spearman's rho	0.01	0.027	0.252**	-0.266**	-0.16	-0.103	-0.042	-0.125	—			
	p-value	0.918	0.768	0.005	0.003	0.08	0.26	0.649	0.186	—			
	N	120	121	121	121	121	121	121	114	—			

Supplementary Table 1 continued

		Percentile	Nutritional status	Enjoyment of food	Food Responsiveness	Slowness in Eating	Satiety Responsiveness	General appetite	Breastfeeding months	Age pregnancy	Birth weight	Breastfeeding	ordinal
Birth weight	Spearman's rho	0.044	0.102	0.09	0.109	0.073	-0.032	0.067	0.036	-0.073	—		
	p-value	0.656	0.295	0.354	0.263	0.452	0.743	0.491	0.71	0.455	—		
	N	107	108	108	108	108	108	108	107	107	—		
Mother schooling	Spearman's rho	0.042	0.034	0.061	-0.041	-0.111	-0.209	0.056	-0.008	0.097	0.019	—	
	p-value	0.646	0.713	0.502	0.658	0.222	0.021	0.542	0.93	0.292	0.841	—	
	N	121	122	122	122	122	122	122	115	121	108	—	
Breastfeeding	Spearman's rho	-0.048	-0.148	0.07	0.256**	0.219	0.056	0.188*	0.796***	-0.116	0.047	0.056	—
	p-value	0.61	0.115	0.46	0.006	0.019	0.549	0.044	<.001	0.22	0.631	0.55	—
	N	114	115	115	115	115	115	115	114	114	107	115	—

Note. * $p < .05$, ** $p < .01$, *** $p < .001$

Supplementary Table 2 - Correlation matrix for the second phase

		Percentile	Nutritional numbers	Enjoyment	Food Responsiveness	Slowness in Eating	Satiety Responsiveness	General appetite	Breastfeeding months	Age pregnancy	Birth weight	Breastfeeding ordinal
Percentile	Spearman's rho	—										
	p-value	—										
	N	—										
Nutritional numbers	Spearman's rho	0.894***	—									
	p-value	< .001	—									
	N	90	—									
Enjoyment	Spearman's rho	-0.04	-0.003	—								
	p-value	0.712	0.976	—								
	N	90	94	—								
Food Responsiveness	Spearman's rho	-0.031	-0.014	-0.074	—							
	p-value	0.771	0.894	0.48	—							
	N	90	94	94	—							
Slowness in Eating	Spearman's rho	-0.228*	-0.165	-0.03	0.089	—						
	p-value	0.03	0.111	0.776	0.391	—						
	N	90	94	94	94	—						
Satiety Responsiveness	Spearman's rho	0.007	0.021	-0.341***	-0.024	-0.01	—					
	p-value	0.944	0.843	< .001	0.816	0.923	—					
	N	90	94	94	94	94	—					
General appetite	Spearman's rho	-0.126	-0.098	0.141	0.338***	0.11	-0.256*	—				
	p-value	0.236	0.35	0.174	< .001	0.292	0.013	—				
	N	90	94	94	94	94	94	—				
Breastfeeding months	Spearman's rho	-0.199	-0.218*	0.068	0.224*	0.123	0.181	0.098	—			
	p-value	0.065	0.039	0.526	0.034	0.247	0.088	0.359	—			
	N	87	90	90	90	90	90	90	—			

Supplementary Table 2 continued

		Percentile	Nutritional numbers	Enjoyment	Food Responsiveness	Slowness in Eating	Satiety Responsiveness	General appetite	Breastfeeding months	Age pregnancy	Birth weight	Breastfeeding ordinal
Age pregnancy	Spearman's rho	0.051	0.111	0.228*	-0.208*	-0.127	-0.049	-0.024	-0.116	—		
	p-value	0.634	0.289	0.028	0.046	0.227	0.638	0.823	0.28	—		
	N	89	93	93	93	93	93	93	89	—		
Birth weight	Spearman's rho	0.247*	0.237*	0.062	0.166	-0.004	0.006	-0.007	-0.008	-0.113	—	
	p-value	0.027	0.031	0.576	0.134	0.972	0.96	0.947	0.945	0.313	—	
	N	80	83	83	83	83	83	83	82	82	—	
Breastfeeding ordinal	Spearman's rho	-0.211	-0.226*	0.055	0.262*	0.199	0.127	0.159	0.815***	-0.14	0.023	—
	p-value	0.05	0.032	0.606	0.013	0.059	0.232	0.135	< .001	0.189	0.836	—
	N	87	90	90	90	90	90	90	89	89	82	—

Note. * $p < .05$, ** $p < .01$, *** $p < .001$

Supplementary Table 3 - Correlation matrix for the second phase

	Percentile	EF BEBQ	FR BEBQ	SE BEBQ	SR BEBQ	GA BEBQ	EF	FR	EOE	DD	FF	EUE	SE	SR	Mother schooli ng	Breast- feeding months	Breast- feeding	Nutritional status	Pregnancy age	Birth weight	Lep.	Ghr .
EF BEBQ	-0.046	—																				
N	54	—																				
FR BEBQ	0.132	-0.17	—																			
N	54	60	—																			
SE BEBQ	-0.216	-0.062	0.094	—																		
N	54	60	60	—																		
SR BEBQ	-0.145	-0.37**	0.088	0.099	—																	
N	54	60	60	60	—																	
GA BEBQ	-0.058	0.02	0.365**	0.197	-0.234	—																
N	54	60	60	60	60	—																
EF	0.183	-0.097	0.181	0.017	-0.108	0.075	—															
N	55	60	60	60	60	60	—															
FR	0.246	-0.311*	0.291*	-0.032	0.133	-0.011	0.325*	—														
N	55	60	60	60	60	60	61	—														
EOE	0.059	0.027	0.134	-0.046	0.064	-0.01	0.086	0.513***	—													
N	55	60	60	60	60	60	61	61	—													
DD	-0.053	-0.067	0.025	0.289*	0.094	0.143	-0.124	0.193	0.068	—												
N	55	60	60	60	60	60	61	61	61	—												
FF	-0.107	-0.199	0.002	-0.089	0.129	0.093	-0.011	0.06	-0.073	-0.194	—											
N	55	60	60	60	60	60	61	61	61	61	—											
EUE	-0.106	-0.125	-0.028	-0.001	0.056	-0.103	-0.17	0.175	0	-0.014	-0.172	—										
N	55	60	60	60	60	60	61	61	61	61	61	—										
SE	-0.126	0.004	-0.1	-0.07	-0.174	0.165	0.118	-0.377**	-0.125	-0.207	0.04	-0.113	—									
N	55	60	60	60	60	60	61	61	61	61	61	61	—									
SR	-0.309*	0.045	0.04	0.241	0.062	-0.014	0.015	0.052	-0.018	-0.033	0.373**	-0.386**	-0.142	—								
N	55	60	60	60	60	60	61	61	61	61	61	61	61	—								

Supplementary Table 3 continued

	Percentile	EF BEBQ	FR BEBQ	SE BEBQ	SR BEBQ	GA BEBQ	EF	FR	EOE	DD	FF	EUE	SE	SR	Mother schooli ng	Breast- feeding months	Breast- feeding	Nutritio nal status	Pregnancy age	Birth weight	Lep.	Ghr .
Mother schooling	0.179	0.153	-0.062	-0.125	-0.257*	-0.06	-0.155	-0.145	-0.111	0.131	-0.292*	0.033	0.026	-0.192	—							
N	55	60	60	60	60	60	61	61	61	61	61	61	61	61	—							
Breastfeeding (months)	-0.148	0.085	0.3*	0.19	0.075	0.196	0.049	0.105	0.071	0.226	-0.079	-0.062	-0.239	0.012	-0.049	—						
N	54	58	58	58	58	58	59	59	59	59	59	59	59	59	59	—						
Breas	-0.207	0.098	0.289*	0.312*	0.052	0.238	-0.012	0.015	-0.031	0.071	0.053	-0.154	-0.175	0.167	-0.07	0.798***	—					
N	54	59	59	59	59	59	60	60	60	60	60	60	60	60	60	59	—					
Nutritional status	0.8***	0.011	0.086	-0.225	-0.288*	-0.033	0.246	0.223	0.049	-0.198	-0.014	-0.125	-0.041	-0.042	0.186	-0.253	-0.213	—				
N	55	59	59	59	59	59	60	60	60	60	60	60	60	60	60	58	59	—				
Pregnancy age	-0.027	0.31*	-0.187	-0.106	-0.028	-0.17	0.242	-0.011	0.008	-0.024	-0.123	-0.172	-0.162	0.173	-0.134	-0.027	-0.056	0.088	—			
N	54	59	59	59	59	59	60	60	60	60	60	60	60	60	60	58	59	59	—			
Birth weight	0.139	0.025	0.035	-0.02	0.138	-0.097	-0.19	0.083	-0.02	0.111	-0.143	-0.021	-0.283*	-0.192	-0.136	0.07	0.166	-0.09	-0.045	—		
N	49	53	53	53	53	53	54	54	54	54	54	54	54	54	54	53	54	53	53	—		
Leptin	0.086	-0.038	0.358*	-0.068	0.064	0.066	-0.033	0.017	-0.169	0.063	0.201	0.066	-0.365*	-0.045	-0.271	0.387*	0.364*	-0.082	-0.093	0.223	—	
N	37	38	38	38	38	38	38	38	38	38	38	38	38	38	38	37	37	38	37	33	—	
Ghrelin	-0.405*	0.334*	-0.086	-0.054	-0.005	-0.104	-0.192	-0.186	0.018	0.102	-0.181	0.13	-0.081	-0.086	-0.04	0.206	0.101	0.428**	-0.025	0.278	0.023	—
N	37	38	38	38	38	38	38	38	38	38	38	38	38	38	38	37	37	38	37	33	38	—

Note. * p < .05, ** p < .01, *** p < .001

values in bold fonts – Pearson's correlations

EF	Enjoyment of Food	EF BEBQ	Enjoyment of food
FR	Food Responsiveness	FR BEBQ	Food Responsiveness
EOE	Emotional Overeating	SE BEBQ	Slowness in eating
DD	Desire to Drink	SR BEBQ	Satiety Responsiveness
FF	Food Fussiness		
EUE	Emotional Undereating		
SE	Slowness in Eating		
SR	Satiety Responsiveness		

3 CONCLUSÃO

Os resultados encontrados permitem concluir que:

O apetite durante a amamentação, de acordo com o BEBQ, foi similar entre crianças com eutrofia e com sobrepeso/obesidade, com exceção a subescala lentidão na alimentação, indicando menor interesse por alimento pelas crianças eutróficas.

O comportamento alimentar, de acordo com o CEBQ, foi similar entre crianças com eutrofia e com sobrepeso/obesidade.

O estado nutricional mais frequente nas três fases foi o eutrófico. Na primeira fase do estudo, 39,4% das crianças avaliadas apresentavam diagnóstico nutricional de sobrepeso/obesidade, 41% na segunda fase e 30% na terceira fase.

As crianças que foram amamentadas ≥ 6 meses permaneceram eutróficas ao longo do tempo, sugerindo um efeito protetor do aleitamento materno.

Crianças com sobrepeso/obesidade apresentaram valores de grelina salivar significativamente mais baixos do que crianças com peso normal, demonstrando que quanto menor os valores de grelina maior o percentil ou estado nutricional. Para a leptina, não houve diferença significativa entre os grupos.

A frequência encontrada de sobrepeso e obesidade no âmbito geral do estudo sugerem alimentação não saudável desde os primeiros anos de vida.

REFERÊNCIAS

- ABESO – Associação Brasileira para o estudo da obesidade e da síndrome metabólica. Diretrizes brasileiras de obesidade. 4.ed. São Paulo: ABESO; 2016 [acesso em 2022 abr 2]. Disponível em: <https://abeso.org.br/diretrizes>.
- Agras WS, Kraemer HC, Berkowitz RI, Hammer LD. Influence of early feeding style on adiposity at 6 years of age. *J Pediatr*. 1990 May;116(5):805-9. doi: 10.1016/s0022-3476(05)82677-0.
- Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, et al. Role of leptin in the neuroendocrine response to fasting. *Nature*. 1996 Jul;382(6588):250-2. doi: 10.1038/382250a0.
- Al-Hamad AH, Al-Naseeb AM, Al-Assaf MS, Al-Obaid SA, Al-Abdulkarim BS, Olszewski PK. Preliminary Exploration of Obesity-Related Eating Behaviour Patterns in a Sample of Saudi Preschoolers Aged 2-6 Years through the Children's Eating Behaviour Questionnaire. *Nutrients*. 2021 Nov;13(11):4156. doi: 10.3390/nu13114156.
- Ardic C, Usta O, Omar E, Yıldız C, Memis E. Effects of infant feeding practices and maternal characteristics on early childhood obesity. *Arch Argent Pediatr*. 2019 Feb;117(1):26-33. doi: 10.5546/aap.2019.eng.26.
- Arslan N, Erdur B, Aydin A. Hormones and cytokines in childhood obesity. *Indian Pediatr*. 2010 Oct;47(10):829-39. doi: 10.1007/s13312-010-0142-y.
- Aydin S, Halifeoglu I, Ozercan IH, Erman F, Kilic N, Aydin S, et al. A comparison of leptin and ghrelin levels in plasma and saliva of young healthy subjects. *Peptides*. 2005 Apr;26(4):647-52. doi: 10.1016/j.peptides.2004.11.008.
- Cunha MM, Moraes VT, Gislayne Castro Nieto. Aleitamento materno: qual a sua relação com a obesidade infantil? *J Paranaense Pediatr*. 2021;22(1):1-4. doi: 10.5935/1676-0166.20210021.
- Enriori PJ, Evans AE, Sinnayah P, Cowley MA. Leptin resistance and obesity. *Obesity (Silver Spring)*. 2006 Aug;14 Suppl 5:254S-258S. doi: 10.1038/oby.2006.319.

Facey A, Dilworth L, Irving R. A review of the leptin hormone and the association with obesity and diabetes mellitus. *J Diabetes Metab*. 2017;8:727. doi: 10.4172/2155-6156.1000727.

Flodmark CE, Lissau I, Moreno LA, Pietrobelli A, Widhalm K. New insights into the field of children and adolescents' obesity: the European perspective. *Int J Obes Relat Metab Disord*. 2004 Oct;28(10):1189-96. doi: 10.1038/sj.ijo.0802787.

Francis RC. Epigenética. Como a ciência está revolucionando o que sabemos sobre hereditariedade. Rio de Janeiro: Zahar; 2015.190p.

Gallant AR, Tremblay A, Périusse L, Bouchard C, Després JP, Drapeau V. The Three-Factor Eating Questionnaire and BMI in adolescents: results from the Québec family study. *Br J Nutr*. 2010 Oct;104(7):1074-9. doi: 10.1017/S0007114510001662.

Giugliano R, Melo AL. Diagnóstico de sobrepeso e obesidade em escolares: utilização do índice de massa corporal segundo padrão internacional. *J Pediatr (Rio J)*. 2004 Mar-Apr;80(2):129-34.

Guzel S, Yibar A, Belenli D, Cetin I, Tanriverdi M. The concentrations of adipokines in goat milk: relation to plasma levels, inflammatory status, milk quality and composition. *J Vet Med Sci*. 2017 Mar 23;79(3):602-7. doi: 10.1292/jvms.16-0061.

Guzmán A, Navarro E, Obando L, Pacheco J, Quirós K, Vásquez L, et al. Effectiveness of interventions for the reversal of a metabolic syndrome diagnosis: An update of a meta-analysis of mixed treatment comparison studies. *Biomedica*. 2019 Dec;39(4):647-62. doi: 10.7705/biomedica.4684.

Ibrahim Abdalla MM. Ghrelin - Physiological Functions and Regulation. *Eur Endocrinol*. 2015 Aug;11(2):90-5. doi: 10.17925/EE.2015.11.02.90.

Jensen SM, Ritz C, Ejlerskov KT, Mølgaard C, Michaelsen KF. Infant BMI peak, breastfeeding, and body composition at age 3 y. *Am J Clin Nutr*. 2015 Feb;101(2):319-25. doi: 10.3945/ajcn.114.092957.

Klok MD, Jakobsdottir S, Drent ML. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. *Obes Rev*. 2007 Jan;8(1):21-34. doi: 10.1111/j.1467-789X.2006.00270.x.

Li BB, Chen ZB, Li BC, Lin Q, Li XX, Li SL, et al. Expression of ghrelin in human salivary glands and its levels in saliva and serum in Chinese obese children and adolescents. *Arch Oral Biol*. 2011 Apr;56(4):389-94. doi: 10.1016/j.archoralbio.2010.10.014.

Li R, Fein SB, Grummer-Strawn LM. Association of breastfeeding intensity and bottle-emptying behaviors at early infancy with infants' risk for excess weight at late infancy. *Pediatrics*. 2008 Oct;122 Suppl 2:S77-84. doi: 10.1542/peds.2008-1315j.

Livingstone MB, Robson PJ. Measurement of dietary intake in children. *Proc Nutr Soc*. 2000 May;59(2):279-93. doi: 10.1017/s0029665100000318.

Llewellyn CH, van Jaarsveld CH, Johnson L, Carnell S, Wardle J. Development and factor structure of the Baby Eating Behaviour Questionnaire in the Gemini birth cohort. *Appetite*. 2011 Oct;57(2):388-96. doi: 10.1016/j.appet.2011.05.324.

Lubkowska A, Radecka A, Bryczkowska I, Rotter I, Laszczyńska M, Dudzińska W. Serum Adiponectin and Leptin Concentrations in Relation to Body Fat Distribution, Hematological Indices and Lipid Profile in Humans. *Int J Environ Res Public Health*. 2015 Sep;12(9):11528-48. doi: 10.3390/ijerph120911528.

Makris MC, Alexandrou A, Papatsoutsos EG, Malietzis G, Tsilimigras DI, Guerron AD, et al. Ghrelin and Obesity: Identifying Gaps and Dispelling Myths. A Reappraisal. *In Vivo*. 2017 Nov-Dec;31(6):1047-1050. doi: 10.21873/invivo.11168.

Mehrdad M, Doaei S, Gholamalizadeh M, Eftekhari MH. The association between FTO genotype with macronutrients and calorie intake in overweight adults. *Lipids Health Dis*. 2020 Aug;19(1):197. doi: 10.1186/s12944-020-01372-x.

Miljković M, Šaranac L, Bašić J, Ilić M, Djindjić B, Stojiljković M, Kocić G, et al. Evaluation of ghrelin and leptin levels in obese, lean and undernourished children. *Vojnosanit Pregl*. 2017;74(10): 963–9.

Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, et al. A role for ghrelin in the central regulation of feeding. *Nature*. 2001 Jan;409(6817):194-8. doi: 10.1038/35051587.

Nascimento VG, da Silva JP, Ferreira PC, Bertoli CJ, Leone C. Maternal breastfeeding, early introduction of non-breast milk, and excess weight in preschoolers. *Rev Paul Pediatr*. 2016 Dec;34(4):454-9. doi: 10.1016/j.rpped.2016.05.004.

Pauli, Patricia Seibert. Associação entre aleitamento materno e excesso de peso em crianças e adolescentes, uma revisão de literatura. Rio Grande do Sul, 2013. *Pediatrics*. 2014 Sep;134 Suppl 1:S50-5. doi: 10.1542/peds.2014-0646I.

Perrine CG, Galuska DA, Thompson FE, Scanlon KS. Breastfeeding duration is associated with child diet at 6 years. *Pediatrics*. 2014 Sep;134 (Suppl 1):S50-5. doi: 10.1542/peds.2014-0646I.

Pietrobelli A, Agosti M; MeNu Group. Nutrition in the First 1000 Days: Ten Practices to Minimize Obesity Emerging from Published Science. *Int J Environ Res Public Health*. 2017 Dec;14(12):1491. doi: 10.3390/ijerph14121491.

Qiao J, Dai LJ, Zhang Q, Ouyang YQ. A Meta-Analysis of the Association Between Breastfeeding and Early Childhood Obesity. *J Pediatr Nurs*. 2020 Jul-Aug;53:57-66. doi: 10.1016/j.pedn.2020.04.024.

Rodrigues L, Espanca R, Costa AR, Antunes CM, Pomar C, Capela-Silva F et al. Association between Salivary Leptin Levels and Taste Perception in Children. *J Nutr Metab*. 2017;2017:7260169. doi: 10.1155/2017/7260169.

Saldiva SR, Venancio SI, de Santana AC, da Silva Castro AL, Escuder MM, Giugliani ER. The consumption of unhealthy foods by Brazilian children is influenced by their mother's educational level. *Nutr J*. 2014 Apr;13:33. doi: 10.1186/1475-2891-13-33.

Sleddens EF, Kremers SP, Thijs C. The children's eating behaviour questionnaire: factorial validity and association with Body Mass Index in Dutch children aged 6-7. *Int J Behav Nutr Phys Act*. 2008 Oct 20;5:49. doi: 10.1186/1479-5868-5-49.

Sociedade Brasileira de Pediatria. Avaliação Nutricional da Criança e do Adolescente: Manual de Orientação. Departamento de Nutrologia. São Paulo: SBP; 2009.

Stunkard AJ, Berkowitz RI, Schoeller D, Maislin G, Stallings VA. Predictors of body size in the first 2 y of life: a high-risk study of human obesity. *Int J Obes Relat Metab Disord*. 2004 Apr;28(4):503-13. doi: 10.1038/sj.ijo.0802517.

Sudi KM, Gallistl S, Tafeit E, Möller R, Borkenstein MH. The relationship between different subcutaneous adipose tissue layers, fat mass and leptin in obese children and adolescents. *J Pediatr Endocrinol Metab*. 2000 May;13(5):505-12. doi: 10.1515/jpem.2000.13.5.505.

Syrad H, van Jaarsveld CH, Wardle J, Llewellyn CH. The role of infant appetite in extended formula feeding. *Arch Dis Child*. 2015 Aug;100(8):758-62. doi: 10.1136/archdischild-2014-307683.

Viana V, Sinde S, Saxton JC. Children's Eating Behaviour Questionnaire: associations with BMI in Portuguese children. *Br J Nutr*. 2008 Aug;100(2):445-50. doi: 10.1017/S0007114508894391.

Wardle J, Guthrie CA, Sanderson S, Rapoport L. Development of the Children's Eating Behaviour Questionnaire. *J Child Psychol Psychiatry*. 2001 Oct;42(7):963-70. doi: 10.1111/1469-7610.00792.

Wasim M, Awan FR, Najam SS, Khan AR, Khan HN. Role of Leptin Deficiency, Inefficiency, and Leptin Receptors in Obesity. *Biochem Genet*. 2016 Oct;54(5):565-72. doi: 10.1007/s10528-016-9751-z.

Webber L, Hill C, Saxton J, Van Jaarsveld CH, Wardle J. Eating behaviour and weight in children. *Int J Obes (Lond)*. 2009 Jan;33(1):21-8. doi: 10.1038/ijo.2008.219.

WHO. World Health Organization. Obesity: preventing and managing the global epidemic - report of a WHO consultation on obesity. Geneva: WHO; 2021.

WHO. World Health Organization. Obesity and overweight. Geneva: WHO; 2021 Jun 9. Disponível em: <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight>.

WHO. World Health Organization. The WHO Child Growth Standards. <https://www.who.int/tools/child-growth-standards>.

ANEXOS

Anexo 1 – Dados demográficos e história social e anamnese

FICHA CLÍNICA – 1ª Avaliação –

Nº DO VOLUNTÁRIO: _____

DATA ____/____/____

NOME _____

DA _____

CRECHE: _____

SALA _____ INTEGRAL ☐ PARCIAL ☐ MANHÃ ☐ TARDE ☐

1. IDENTIFICAÇÃO E DADOS SOCIOECONOMICOS

Nome da criança

Data de nascimento ____/____/____ Idade ____ meses. Sexo: () M () F

Endereço _____ Nº _____ Bairro: _____

Cidade: _____ Telefone _____

Nome do Pai

Data de nascimento: ____/____/____ Idade ____ anos

Grau de instrução: ☐ sem escolaridade ☐ fundamental ☐ médio ☐ superior

Estudou até que série? _____

Profissão _____ Fone _____

Nome da Mãe

Data de nascimento: ____/____/____ Idade ____ anos

Grau de instrução: ☐ sem escolaridade ☐ fundamental ☐ médio ☐ superior

Estudou até que série? _____

Profissão _____ Fone _____

Tem outros Irmãos () Não () Sim Quantos? _____

Qual é a ordem da criança na família? () Primogênito () Meio () Caçula () Outros: _____

Renda familiar R\$ _____

Criança mora com: () Mãe e Pai () Mãe () Pai () Outros: _____

Possuem automóvel ? () SIM () NÃO

Plano de saúde? () SIM () NÃO

ANAMNESE

História PRÉ NATAL

A mãe teve alguma alteração durante a gravidez? () Não () Sim. Qual? _____

A mãe utilizou algum remédio na gravidez? () Não () Sim. Qual? _____

A mãe fumou durante a gravidez? () Não () Sim

A mãe usou droga durante a gravidez? () Não () Sim

A mãe utilizou flúor durante a gravidez? () Não () Sim. Qual? _____

A mãe teve orientação odontológica durante a gravidez? () Não () Sim

Idade em que a mãe engravidou: _____

Peso antes da gravidez: _____ Peso grávida _____

A mãe praticou exercícios físicos durante a gravidez? () Não () Sim Quais? _____ Com que frequência? _____

Trabalhou até que mês? _____

Realizou Pré-Natal ? _____

HISTÓRIA NATAL

PARTO Normal ☐ Fórceps ☐ Cesariana ☐

Houve alguma complicação durante o parto? () SIM () NÃO Qual ? _____

NASCIMENTO

Sua gestação durou quantas semanas _____ O bebê nasceu prematuro ? () SIM () NÃO

Qual foi o peso ao nascer? _____

Nasceu com algum problema de saúde ? () Sim () Não

Qual? _____

Teve algum problema de saúde grave () Sim () Não Qual? _____

ALIMENTAÇÃO PRIMEIROS 6 MESES

Seu bebê foi amamentado no peito ? () Sim () Não Por quantos meses ele foi amamentado ? _____

Quantas mamadas durante o dia ? _____ Quantas mamadas durante a noite ? _____

Até que idade? _____

Se seu bebê não foi amamentado no peito , por qual motivo ele não foi amamentado no peito ?

Seu bebê está sendo amamentado no peito atualmente / Hoje ? () Sim () Não

ALEITAMENTO ATUAL ARTIFICIAL:

Introduziu leite artificial () Não () Sim Motivo: _____

Idade: _____ Ainda usa leite artificial ou fórmula? _____

Tipo de leite: () vaca () cabra () soja () fórmula

Uso: () adoçante () açúcar () mel () Toddy ou Nescau () Mucilon () Outros: _____

A criança bebe leite em: () mamadeira () copo () Ambos

Seu bebê tem alergia a lactose ? () Sim () Não

Se usa mamadeira, responder as questões abaixo:

Quantas mamadeiras por dia ? _____ Quantas mamadeiras a noite ? _____

A criança dorme tomando a mamadeira? () Não () Sim

Você aumenta o furo da mamadeira ? () Sim () Não Porque? _____

O esvaziamento da mamadeira ocorre: () rápido () devagar () normal

Bebe outros líquidos em: () mamadeira () copo () ambos

Tem horário regular para as refeições () Não () Sim

Idade que começou a comer frutas e tomar sopa _____

Considera a alimentação de seu filho equilibrada: () sim () não

Como é o apetite da criança: () normal () tem bastante apetite () tem pouco apetite

Preferência de alimentos: () líquidos () pastosos () semi-sólidos () sólidos () todos

Preferência de alimentos: () doce () salgado

Durante as refeições toma algum líquido junto ? () SIM () NÃO Qual ? _____

Você acha que seu filho mastiga bem os alimentos ? () Sim () Não

Seu bebê tem alergia a alguma alimento ? () Sim () Não Qual ? _____

HISTÓRIA NEO-NATAL

Seu bebê teve algum problema durante o primeiro mês de vida ? () Sim () Não Qual ? _____

☐ Icterícia

☐ Dificuldades respiratórias

☐ Febre alta

☐ Dificuldades de alimentação

☐ Doenças graves

☐ Dentes do nascimento ao 1º mês

Seu bebê tem alguma doença ? () Sim () Não Qual ? _____

Seu bebê toma alguma medicação ? () Sim () Não Qual ? _____

Seu bebê tem alergia a alguma medicação ? () Sim () Não Qual ? _____

As vacinas estão em dia? () SIM () NÃO Quais estão faltando

? _____

Posto de saúde onde é atendido _____ Nome do médico

responsável: _____

Data do último exame médico: ____/____/____

Hábitos Deletérios

HÁBITOS – Fazer um X nas opções abaixo.

TIPO			FREQUÊNCIA			ÀS VEZES
	SIM	NÃO	DIA	NOITE	CONTÍNUO	
Sucção de chupeta						
Sucção dos dedos						
Sucção de paninho						
Sucção dos lábios						
Mordedura dos lábios						
Onicofagia (roer unhas)						

Se faz uso de chupeta responder as questões abaixo:

Marca ou tipo de chupeta _____

Idade em que começou a usar a chupeta ? _____

HÁBITOS DE HIGIENE BUCAL DO BEBÊ SEM DENTES

Você higieniza a boca do seu bebê? () Não () Sim Com que frequência? _____

Você usa o que para fazer essas limpeza? _____

Hábitos de Higiene Bucal do bebê COM DENTES

Com que idade você começou a escovar os dentes do seu filho(a)? () desde sempre () primeiro dente () outros

Quantas vezes por dia você escova os dentes de seu filho ? _____

O que você usa para higiene bucal do seu filho ? ☐ escova ☐ fio dental ☐ outros (bochechos)

Responsável pela escovação ☐ mãe ☐ pai ☐ criança ☐ criança e mãe

Qual pasta de dente usada? ☐ com flúor ☐ sem fluor ☐ não sabe

Você sempre usou paste de dente fluoretada: ☐ sim ☐ não

Receberam orientação sobre higiene bucal ☐ sim ☐ não

Quem forneceu essas orientações ? _____

Dentadura decídua

Já nasceu algum dente em seu bebê? _____ Com quantos meses esse dente nasceu ? _____

Qual dente foi ? _____ Teve sintomas? () Não () Sim

Quais? _____

Anexo 2 – Saúde bucal e diagnóstico de cárie**SAÚDE BUCAL E DIAGNÓSTICO DE CÁRIE 1ª Avaliação****ODONTOGRAMA**

Nome: _____ Nº: _____

Data nasc.: _____ Idade (meses): _____ Sexo: (F) (M): Cor: (B) (N) (P)

Creche: _____

INTEGRAL ☐**PARCIAL** ☐ **MANHÃ** ☐ **TARDE** ☐

0- Sadio

1- Cárie ativa (superfície intacta)

2- Cárie ativa (superfície descontínua)

3- Cárie ativa (cavidade)

4- Cárie inativa (superfície intacta)

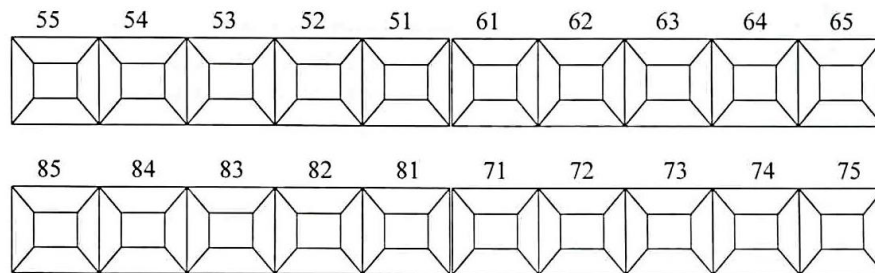
5- Cárie inativa (superfície descontínua)

6- Cárie inativa (cavidade)

7- Restaurada (superfície sadia)

8- Restaurada + cárie ativa

9- Restaurada + cárie inativa



Valor do ceo-d: _____

Valor de ceo-s: _____

Agnesias

Hipoplasias

Trauma

Gengivite

0

1

Biofilme Visível

0

1

Anexo 3 – Avaliação antropométrica

AVALIAÇÃO ANTROPOMÉTRICA

1ª Avaliação Data: _____

Peso (kg): _____ Estatura(cm): _____ IMC(kg/m²): _____ DN: _____

2ª Avaliação Data: _____

Peso (kg): _____ Estatura(cm): _____ IMC(kg/m²): _____ DN: _____

3ª Avaliação Data: _____

Peso (kg): _____ Estatura(cm): _____ IMC(kg/m²): _____ DN: _____

Valores de referência para diagnóstico do estado nutricional.

Valor encontrado na criança		Diagnóstico nutricional
< Percentil 0,1	< Escore z -3	Magreza acentuada
≥ Percentil 0,1 e < Percentil 3	≥ Escore z -3 e < Escore z -2	Magreza
≥ Percentil 3 e < Percentil 85	≥ Escore z -2 e < Escore z +1	Eutrofia
≥ Percentil 85 e < Percentil 97	≥ Escore z +1 e < Escore z +2	Sobrepeso
≥ Percentil 97 e < Percentil 99,9	≥ Escore z +2 e < Escore z +3	Obesidade

Anexo 4 - Questionário do comportamento alimentar do bebê (BEBQ)

BABY EATING BEHAVIOUR QUESTIONNAIRE (BEBQ) These questions are about your baby's appetite over his/her first few months of life. We are specifically interested in the period during which your baby is fed milk only, i.e. no solid foods or pre-prepared baby food yet.						
How would you describe your baby's feeding style at a <u>typical daytime feed</u> ?						
	Never	Rarely	Sometimes	Often	Always	
1. My baby seemed contented while feeding	☐	☐	☐	☐	☐	EF
2. My baby frequently wanted more milk than I provided	☐	☐	☐	☐	☐	FR
3. My baby loved milk	☐	☐	☐	☐	☐	EF
4. My baby had a big appetite	☐	☐	☐	☐	☐	GA
5. My baby finished feeding quickly*	☐	☐	☐	☐	☐	SE
6. My baby became distressed while feeding*	☐	☐	☐	☐	☐	EF
7. My baby got full up easily	☐	☐	☐	☐	☐	SR
8. If allowed to, my baby would take too much milk	☐	☐	☐	☐	☐	FR
9. My baby took more than 30 minutes to finish feeding	☐	☐	☐	☐	☐	SE
10. My baby got full before taking all the milk I think he/she should have	☐	☐	☐	☐	☐	SR
11. My baby fed slowly	☐	☐	☐	☐	☐	SE
12. Even when my baby had just eaten well he/she was happy to feed again if offered	☐	☐	☐	☐	☐	FR
13. My baby found it difficult to manage a complete feed	☐	☐	☐	☐	☐	SR
14. My baby was always demanding a feed	☐	☐	☐	☐	☐	FR
15. My baby sucked more and more slowly during the course of a feed	☐	☐	☐	☐	☐	SE
16. If given the chance, my baby would always be feeding	☐	☐	☐	☐	☐	FR
17. My baby enjoyed feeding time	☐	☐	☐	☐	☐	EF
18. My baby could easily take a feed within 30 minutes of the last one	☐	☐	☐	☐	☐	FR

* Items need to be reversed for scoring

SCORING OF THE BEBQ

(Never=1, Rarely=2, Sometimes=3, Often=4, Always=5)

Food responsiveness (FR) = item mean FR

Enjoyment of food (EF) = item mean EF

Satiety responsiveness (SR) = item mean SR

Slowness in eating (SE) = item mean SE

General appetite (GA) = single item that
measures overall/
general appetite

*Reversed items (5 & 6)

Anexo 5 - Questionário do Comportamento Alimentar de Crianças (CEBQ)

Child Eating Behaviour Questionnaire (CEBQ)

Please read the following statements and tick the boxes most appropriate to your child's eating behaviour.

	Never	Rarely	Some -times	Often	Always	
My child loves food	☐	☐	☐	☐	☐	EF
My child eats more when worried	☐	☐	☐	☐	☐	EOE
My child has a big appetite	☐	☐	☐	☐	☐	SR*
My child finishes his/her meal quickly	☐	☐	☐	☐	☐	SE*
My child is interested in food	☐	☐	☐	☐	☐	EF
My child is always asking for a drink	☐	☐	☐	☐	☐	DD
My child refuses new foods at first	☐	☐	☐	☐	☐	FF
My child eats slowly	☐	☐	☐	☐	☐	SE
My child eats less when angry	☐	☐	☐	☐	☐	EUE
My child enjoys tasting new foods	☐	☐	☐	☐	☐	FF*
My child eats less when s/he is tired	☐	☐	☐	☐	☐	EUE
My child is always asking for food	☐	☐	☐	☐	☐	FR
My child eats more when annoyed	☐	☐	☐	☐	☐	EOE
If allowed to, my child would eat too much	☐	☐	☐	☐	☐	FR
My child eats more when anxious	☐	☐	☐	☐	☐	EOE
My child enjoys a wide variety of foods	☐	☐	☐	☐	☐	FF*
My child leaves food on his/her plate at the end of a meal	☐	☐	☐	☐	☐	SR
My child takes more than 30 minutes to finish a meal	☐	☐	☐	☐	☐	SE

SCORING OF THE CEBQ

(Never=1, Rarely=2, Sometimes=3, Often=4, Always=5)

Food responsiveness = item mean FR

Emotional over-eating = item mean EOE

Enjoyment of food = item mean EF

Desire to drink = item mean DD

Satiety responsiveness = item mean SR

Slowness in eating = item mean SE

Emotional under-eating = item mean EUE

Food fussiness = item mean FF

*Reversed items

Anexo 6 – Relatório de verificação de Originalidade e prevenção de plágio

Tese de Doutorado - Gabriela Novo Borghi			
RELATÓRIO DE ORIGINALIDADE			
20%	18%	9%	4%
ÍNDICE DE SEMELHANÇA	FONTES DA INTERNET	PUBLICAÇÕES	DOCUMENTOS DOS ALUNOS
FONTES PRIMÁRIAS			
1	repositorio.unicamp.br Fonte da Internet	12%	
2	Ana Freitas, Gabriela Albuquerque, Cláudia Silva, Andreia Oliveira. "Appetite-Related Eating Behaviours: An Overview of Assessment Methods, Determinants and Effects on Children's Weight", Annals of Nutrition and Metabolism, 2018 Publicação	1%	
3	"Abstracts", Public Health Nutrition, 2013 Publicação	1%	
4	scindeks-clanci.ceon.rs Fonte da Internet	1%	
5	hdl.handle.net Fonte da Internet	1%	
6	Vladimir Shusterman, Charles F. McTiernan, Anna Goldberg, Samir Saba, Guy Salama, Barry London. "Adrenergic stimulation promotes T-wave alternans and arrhythmia inducibility in a TNF- α genetic mouse model of	1%	

Anexo 7 – Comitê de Ética

Título da Pesquisa: Condições bucais, aspectos nutricionais, biomarcadores salivares do apetite e desenvolvimento da dentição decídua.
Pesquisador Responsável: Gabriela Novo Borghi
Área Temática:
Versão: 3
CAAE: 55088216.9.0000.5418
Submetido em: 17/11/2016
Instituição Proponente: Faculdade de Odontologia de Piracicaba - Unicamp
Situação da Versão do Projeto: Aprovado
Localização atual da Versão do Projeto: Pesquisador Responsável
Patrocinador Principal: Financiamento Próprio



Comprovante de Recepção:  PB_COMPROVANTE_RECEPCAO_827683

Anexo 8 – Comprovante de submissão do artigo

● Journal of Pediatric Nursing

Confirming submission to Journal of Pediatric Nursing

Para: Maria BEATRIZ Gavião,

Responder A: Journal of Pediatric Nursing

Ontem 16:58



This is an automated message.

Overweight and obesity, food behavior and salivary appetite biomarkers in early childhood: a longitudinal study

Dear Professor Gavião,

We have received the above referenced manuscript you submitted to Journal of Pediatric Nursing.

To track the status of your manuscript, please log in as an author at <https://www.editorialmanager.com/jpediatr Nurs/>, and navigate to the "Submissions Being Processed" folder.

Thank you for submitting your work to this journal.

Kind regards,
Journal of Pediatric Nursing

More information and support

You will find information relevant for you as an author on Elsevier's Author Hub: <https://www.elsevier.com/authors>

FAQ: How can I reset a forgotten password?

https://service.elsevier.com/app/answers/detail/a_id/28452/supporthub/publishing/kw/editorial+manager/

For further assistance, please visit our customer service site: <https://service.elsevier.com/app/home/supporthub/publishing/>. Here you can search for solutions on a range of topics, find answers to frequently asked questions, and learn more about Editorial Manager via interactive tutorials. You can also talk 24/7 to our customer support team by phone and 24/7 by live chat and email.

This journal uses the Elsevier Article Transfer Service. This means that if an editor feels your manuscript is more suitable for an alternative journal, then you might be asked to consider transferring the manuscript to such a journal. The recommendation might be provided by a Journal Editor, a dedicated Scientific Managing Editor, a tool assisted recommendation, or a combination. For more details see the journal guide for authors.

#AU_JPEDIATR NURS#

To ensure this email reaches the intended recipient, please do not delete the above code

In compliance with data protection regulations, you may request that we remove your personal registration details at any time. (Use the following URL: <https://www.editorialmanager.com/jpediatr Nurs/login.asp?a=r>). Please contact the publication office if you have any questions.